SCIENTIFIC AGRICULTURE

Vol. XXI FEBRUARY, 1941 No. 6

THE RELATIVE VALUES OF SEED INJURED BY RUST, FROST OR DROUGHT[1]

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[Received for publication June 7, 1940]

As frequently happens, adverse conditions such as the presence of rust, drought, inclement harvesting weather, or the occurrence of early frosts may reduce the plumpness and affect the colour of grain. Opinions as to the relative values of such grain for seeding purposes have differed considerably, and many experiments have been conducted in attempts to settle this problem. For the most part, the work has been confined to laboratory tests related to germination, although a few investigators have endeavoured to relate laboratory tests with field results. The material used in most cases has been ordinary commercial seed grain, pedigreed varieties not being used.

Each season, it is necessary for the Plant Products Division, Production Service, Dominion Department of Agriculture, to establish minimum standards for the different seed grades. Where pedigreed seed is involved, it has been difficult to balance the special value of such seed on the one hand, with the effects of different degrees of damage suffered by this seed as a result of rust, frost or drought on the other. Unpedigreed commercial seed might consist of one or several varieties which reasonably might have as many dates of maturity. Obviously pedigreed seed provides better material for the solution of the present problem.

It was with a view to obtaining further data on this question that the present study was initiated some years ago by the Plant Products Division, Production Service, in co-operation with the Cereal Division of the Central Experimental Farm, Dominion Department of Agriculture, Ottawa, Ont.

Previous Work

As already indicated, much work has been done in an attempt to evaluate seed which has been injured in various ways. The seed value of *frosted* wheat was carefully reviewed by Lewis and McCalla (2) in 1937. These authors studied the effect on germination, field survival and yield. The material used was composed of samples received from farmers whose crops had been frosted in 1928 or 1930, or samples artificially frozen at the University of Alberta in 1930–32. It was shown that the yields obtained from frozen seed were satisfactory, even with poor laboratory or field germination, when sown on clean land and under favourable conditions for growth. Under adverse conditions, on the other hand, the results might be quite the reverse.

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The literature dealing with the effects of stem rust on the seed value of grain was reviewed by Mead (3) early in 1939. His investigation involved laboratory tests conducted on commercial seed available after the severe rust epidemic of 1935. He recommended that, where it was necessary to use shrivelled seed, heavy cleaning of the grain should be followed, and the best of this grain sown at the usual rate, but as shallow as moisture conditions would permit. A field survey conducted during the following summer did not indicate any significant difference in the crops produced from plump and shrivelled seed.

MATERIALS AND METHODS

In order to eliminate, as far as possible, any factors introduced by differences in variety, a strain of registered Marquis known as "10 B", developed by Dr. Seager Wheeler of Rosthern, Sask., was used for the experiment. Ten pound samples of seed of this variety, representing in each case material injured by rust, frost, drought and inclement weather were procured from the 1933 crop, by the Plant Products Division, from growers of registered seed of this variety in Saskatchewan and Alberta. As originally planned, the test was to have been conducted each year, for three years, at the Experimental Farms at Indian Head, Sask., Brandon, Man., and Ottawa, Ont., and new seed of each class of injury was to be secured annually. It was not found possible, however, in 1933 and 1934, to obtain a complete set of suitable samples, consequently blanks were left in the test which spoiled the continuity of the experiment. It was therefore decided to secure sufficient seed from the 1935 crop to conduct the test with the same seed for three years. A very complete set of samples, providing all the degrees of injury and varying degrees of plumpness desired, was procured as follows:

SOURCE OF SEED AND PHYSICAL PROPERTIES OF SAMPLES USED IN THE TEST

(All 10B strain of Marquis wheat)

Sources of seed	Physical properties of kernels
1. University of Alberta, Edmonton	Plump and Red
2. Dr. E. M. Hall, Edmonton	Plump and Piebald
3. Dr. E. M. Hall, Edmonton	Piebald
4. Dr. E. M. Hall, Edmonton	Frosted
5. Geo. Avery, Kelso, Sask.	Weathered
6. R. S. Hall, Earl Gray, Sask.	Shrivelled
7. J. J. Harrison, Bracken, Sask.	Badly shrivelled
8. Chas. N. Lintott, Raymore, Sask.	Rusted

Description of Samples

The *plump and red* sample, as the name would indicate, was a fine sample with good colour and weighing 63 pounds per bushel, with a 1000 kernel weight of 36 grams.

The *plump and piebald* sample had a smaller sized kernel, weighing 62.5 pounds per bushel, and 34.8 grams per 1000 kernels; it was piebald or starchy in appearance with a few dark red kernels appearing, up to about 5% of the total.

The *piebald* sample was similar to the *plump piebald*, except that it was one and a half *grams* lower in weight than the latter.

The *frosted sample* was of fair size and maturity, but the bran coat was puckered with frost blisters and the sample only weighed 60.5 pounds per bushel and 34 grams per 1000 kernels.

The *weathered sample* had suffered from dry winds and had a bleached appearance weighing only 59.5 pounds and was down to 33 grams per 1000 kernels.

The *shrivelled sample*, as the name would indicate, was small and hollow cheeked, down in bushel and kernel weight to 56.5 lb. and 26.2 grams respectively. This was the only sample in the test with germination below 90.

The *badly shrivelled sample* was a degree worse than the shrivelled, being just ½ pound lower in bushel and 0.2 gram lower in kernel weight.

The *rusted sample* was composed of typical rusted berries, lacking in filling and weight, although with a fair colour and 92% germination. The bushel weight was only 51 lb., while the kernel weight was down to 16 grams per thousand.

In Table 1 (page 310) will be found the weight per bushel, 1000 kernel weight, and germination of the various grades at the start and finish of the experiment.

From Table 1 it is evident that the bushel weight and 1000 kernel weight were maintained, as might be expected, but in the three years of the test, the germination fell away very rapidly. An effort was made to sow, as nearly as possible, 250 seeds per 18½-foot row, and in preparing the seed for sowing, a correction, based on the percentage of germinable seed, was made to compensate for the lower germination. Where the germination was 97% or better, no correction was made; but where the percentages were lower, the following table of corrections was followed:—

Percentage germination	Number of kernels added	Percentage germination	Number of kernels added
97 – 100	0	68 – 72	106
93 – 96	13	63 – 67	134
88 – 92	28	58 – 62	166
83 – 87	43	53 – 57	204
78 – 82	63	48 – 52	248
73 – 77	84	43 – 47	304

While these corrections are never fully satisfactory, an attempt at least was made to bring the number of plants grown up to a common level.

Location of Tests

The tests were conducted at Indian Head, Sask. and Ottawa, in 1936–37 and 38, and at Brandon only for 1936 and 1937. The section dealing with the rate of seeding rusted and plump seed was conducted at the three points mentioned in 1937 and 1938 only.

TABLE 1.—DESCRIPTION OF SEED

Grade	Weight per bu.		1000 kernel wt.		Germination	
	1935	1938	1935	1938	1935	1938
	lb.	lb.	gm.	gm.	%	%
1 Plump and red	63.0	63.0	36.0	36.0	96	67
2 Plump and piebald	62.5	62.5	34.8	34.8	90	66
3 Piebald	62.5	62.5	33.3	33.3	97	53
4 Frosted	60.5	60.5	34.0	34.0	94	25
5 Weathered	59.5	59.5	33.0	33.0	93	43
6 Shrivelled	55.5	56.5*	26.2	26.2	85	53
7 Badly shrivelled	55.0	55.0	26.0	26.0	96	67
8 Rusted	51.0	51.0	16.0	16.0	92	75

* It was impossible to check the bushel weight of this sample, for the 1935 weight.

Preparation of the Material and Method of Analysis

The material was all put up at Ottawa, ready for sowing at the three points; quadruplicate 5 rod row plots, 18½ feet long, and randomized in a modified balanced block system were used. At harvest time the plots were cut down to the rod length by taking a foot from each end, and only the three centre rows were harvested for yield purposes.

In Tables 2 to 5 inclusive will be found the agronomic data obtained from the tests conducted at the Experimental Farms at Indian Head, Sask., Brandon, Man., and Ottawa, Ont.

Only the 3-year averages for "Days to mature" and "Length and strength of straw" are recorded, as the yearly figures were not found to vary sufficiently to warrant reporting.

TABLE 2.—AGRONOMIC DATA FROM THE TEST AT INDIAN HEAD, SASK. FOR YEARS 1936–38 INCLUSIVE

Description of seed used	Days to mature, average 1936–38	Strength of straw, average 1936–38	Length of straw, average 1936–38	Yield per acre in bushels			
				1936	1937	1938	Average
1 Plump and red	95.7	10.0	29.8	26.3	24.9	22.6	24.58
2 Plump and piebald	95.7	10.0	29.3	26.7	25.1	22.7	24.80
3 Piebald	96.0	10.0	29.5	28.0	24.0	20.8	24.26
4 Frosted	96.3	10.0	29.5	25.1	22.9	19.2	22.08
5 Weathered	95.7	10.0	29.5	26.8	23.3	21.6	23.90
6 Shrivelled	96.0	10.0	29.5	25.9	23.5	19.7	23.00
7 Badly shrivelled	95.7	10.0	29.5	26.1	23.7	21.5	23.78
8 Rusted	96.0	10.0	29.0	24.9	20.9	19.6	21.80
Standard Error of Variety Mean				1.54	1.15	.91	
Significant Difference				2.31	1.60	1.38	
Mean Yield in Bushels				26.16	23.60	21.30	
F Value				1.06	3.5	8.0	
5% Point				2.14	2.14	2.14	

On examination of the data from Indian Head, it is evident that there is no appreciable difference presented in the days to mature or in length and strength of straw. In the case of yield, there is no significant difference

between any of the 8 classes of seed used in 1936, but in 1937 a difference does obtain between the yields of plump red seed and the rusted seed, but only between these. In 1938 the yields from frosted seed, shrivelled seed and rusted seed are significantly lower than those from plump, and plump and piebald seed. This may be due in part to the low germination already noted in this sample. In the case of the rusted sample, only a slight reduction in the germination was noted, which would seem to indicate that while there was a relatively small difference in yield, nevertheless the difference is valid in two years out of three.

TABLE 3.—AGRONOMIC DATA FROM THE TEST AT BRANDON, MAN., FOR YEARS 1936–37

Description of seed used	Days to mature, average 1936–37	Strength of straw, average 1936–37	Yield per acre in bushels		
			1936	1937	Ave.
1 Plump and red	88.5	8.0	31.4	19.6	25.5
2 Plump and piebald	88.5	8.1	31.2	20.5	25.9
3 Piebald	88.5	8.0	29.1	20.8	24.9
4 Frosted	89.0	7.5	29.9	15.4	22.7
5 Weathered	88.5	7.8	30.1	20.3	25.2
6 Shrivelled	88.5	7.6	28.6	20.2	24.4
7 Badly shrivelled	89.0	7.5	26.9	20.0	23.5
8 Rusted	89.5	7.1	22.4	17.9	20.2
Standard Error of Variety Mean			2.56	.75	
Mean yield in bushels			29.4	19.9	
Co. of Variability in %			17.4	7.5	
Significant difference			7.68	2.3	
F value			1.21	5.8	
5% point			2.14	2.14	

TABLE 4.—AGRONOMIC DATA FROM THE TEST AT OTTAWA, ONT., FOR THE YEARS 1936–38 INCLUSIVE

Description of seed used	Days to mature, average 1936–38	Strength of straw, average 1936–38	Yield per acre in bushels			
			1936	1937	1938	Ave.
1 Plump and red	91.4	8.6	24.1	17.8	16.8	19.6
2 Plump and piebald	91.1	8.7	23.3	18.2	18.1	19.9
3 Piebald	91.2	8.7	24.6	15.4	15.3	18.4
4 Frosted	91.4	8.6	24.1	14.5	12.5	17.0
5 Weathered	91.4	9.0	24.0	15.3	19.3	19.5
6 Shrivelled	91.3	8.6	24.9	15.5	16.8	19.1
7 Badly shrivelled	91.4	8.3	26.4	17.4	17.7	20.5
8 Rusted	91.3	8.4	20.3	13.5	16.7	16.8
Standard Error of variety mean			2.40	.99	1.31	
Mean yield in bushels			24.50	16.70	17.29	
C. of variability in %			9.79	11.92	7.50	
Significant differences			3.60	2.97	3.93	
F value			1.35	3.40	2.09	
5% point			2.14	2.10	2.14	

In the case of the Brandon Test, there was no valid significance in the 1936 test, but the plump-piebald and piebald samples outyielded the rusted and frosted sample in 1937 by a significant difference.

At Ottawa a significant difference was obtained only in 1937 when the plump-red and plump-piebald samples outyielded the rusted and frosted samples.

TABLE 5.—ORDER OF STANDING BASED ON AVERAGE YIELDS OBTAINED AT THREE SEPARATE POINTS FOR YEARS 1936–37 AND 38

Description of sample	Brandon, Man. av. 2 yrs.	Ind. Head, Sask. av. 3 yrs.	Ottawa, Ont. av. 3 yrs.	Average standing for 8 tests	Order of standing in 8 tests
Plump and piebald	1	1	2	4	1
Plump and red	2	2	3	7	2
Weathered	3	4	4	11	3
Badly shrivelled	6	5	1	12	4
Piebald	4	3	6	13	5
Shrivelled	5	6	5	16	6
Frosted	7	7	7	21	7
Rusted	8	8	8	14	8

Taking the results from all three Stations in their order of standing in Table 5, the evidence would indicate that plump kernels whether piebald or not make superior seed grain from the standpoint of yielding ability to either weathered, very poor, piebald, shrivelled, frosted or rusted grain. This is to be expected in view of the extra supply of plant food in the larger endosperm of a plump berry. This is in line with Grekov (1) who demonstrated the enhanced vegetation, maturity and yield by increasing the supply of endosperm. Weathering does not appear to be harmful to seed grain, provided the germination is on a satisfactory level and that no sprouting has taken place.

THE RELATIVE VALUE OF DIFFERENT RATES OF SEEDING OF PLUMP AND RUSTED GRAIN FOR SEED

Following years when rust has been prevalent, it has been necessary, up to the present, for many farmers to sow rusted seed, whether they desired to use this type of seed or not. In 1935, rust was bad in many areas in Saskatchewan and Manitoba, and with the scarcity of good seed the problem in 1936 was to know *how much seed to sow*, and how to compensate for the much poorer rusted seed, the only material frequently available.

With the excellent material already available, it was thought advisable to include this further test in an effort to obtain a satisfactory answer, if possible, to this pressing problem. Three rates of seeding of plump seed and rusted seed were decided upon, namely at 75, 90 and 105 lb. per acre. The necessary seed was sent to the same three Farms, Indian Head, Brandon and Ottawa for testing. The results obtained will be found in Table 6.

It will be noted that significant differences were obtained only at Indian Head in 1938, and at Ottawa in 1937. Nevertheless, even in tests in which the difference was not significant it will be noted that generally speaking, there was a slight increase in yield as the rates of seeding increased

TABLE 6.—YIELD DATA IN BUSHELS PER ACRE ON RATES OF SEEDING PLUMP AND RUSTED MARQUIS WHEAT AT EXPERIMENTAL FARMS AT BRANDON, INDIAN HEAD AND OTTAWA, 1937–38

Variety	Rate of seeding in lbs.	Brandon, bu. per acre			Indian Head, bu. per acre			Ottawa, bu. per acre			Average three farms		
		1937	1938	Ave.	1937	1938	Ave.	1937	1938	Ave.	1937	1938	Grand average
Marquis E. 32 Check	75	18.6	Crop failure.		19.9	24.4	22.15	14.7	17.1	15.9	17.8	20.7	18.9
Marquis E. 32 Check	90	21.1			18.9	22.8	20.9	20.4	21.3	20.9	20.1	22.0	20.9
Marquis Plump 1312	75	20.2			20.7	22.5	21.6	17.9	18.2	18.0	19.6	20.3	19.9
Marquis Plump 1312	90	19.4			21.8	22.9	22.35	19.6	18.1	18.9	20.3	20.5	20.3
Marquis Plump 1312	105	19.6			21.6	23.6	22.60	20.3	21.3	20.8	20.5	22.4	21.3
Marquis Rusted 3161	75	19.3			18.5	21.8	20.15	18.4	15.7	17.0	18.7	18.7	18.7
Marquis Rusted 3161	90	20.0			18.9	21.4	20.15	20.1	18.5	19.3	19.7	19.9	19.8
Marquis Rusted 3161	105	20.2			17.0	20.7	18.85	20.1	18.9	19.5	19.1	19.8	19.4
Standard Error of Var.		.73			2.52	.46		.84	1.27				
Mean in Bus.		19.8			19.7	22.4		18.91	18.61				
C. of variability in %		10.6			2.56	4.10		8.93	13.58				
Significant Difference		2.19			7.60	1.4		2.53	3.81				
F value		1.09			2.40	6.7		5.26	2.26				
5% Point		2.55			2.55	2.55		2.55	2.62				

in both the plump and rusted seed. On the average of the three tests, *plump* seed in every instance yielded higher than the corresponding rate for *rusted* seed, while the highest rate for the rusted seed did not equal the lowest rate of the *plump* seed.

SUMMARY

Eight samples of the 10B strain of Marquis wheat, representing eight different kinds of physical properties in their kernels, from plump and red, plump and piebald to frosted, weathered and rusted kernels were grown in replicated 5-rod row plots for three years at Ottawa, Ont. and Indian Head, Sask. and for two years at Brandon, Man.

At Indian Head, significant differences were found in the yield between *plump* and *red kernels* over the *rusted* sample in two out of the three years, while at Brandon the *plump* and *piebald* samples outyielded the *rusted* and *frosted* samples by significant differences of 0.3 and 2.8 bushels respectively. In the case of Ottawa, a significant difference was obtained only once in the three years between *plump red* and *plump piebald* as against the *rusted* and *frosted* samples.

In tests to determine the proper rate to sow rusted grain, significant differences were obtained only at Indian Head in 1938 and at Ottawa in 1937, and while significance was not obtained in every test, there was a slight increase in yield as the rates of seeding increased in all tests.

It is concluded from these experiments that *plump seed* is preferable to *rusted seed*, and usually gives somewhat higher yields. If rusted seed is used, a much higher rate of seeding is necessary in order to compensate as far as possible for lack of size and general vigour of such seed.

The general conclusion reached, in analysing these experiments conducted for a 3-year period under a wide range of climatic conditions is that while *plump* well matured, uniform grain of good germination is preferable for seed purposes to *weathered*, *badly shrivelled*, *piebald*, *shrivelled*, *frosted* or *rusted* grain, nevertheless where germination and pedigree are satifactory the yield between the poorest and best quality seed in tests where any significance was obtained was never greater than 4.2 bushels at Indian Head, 2.6 bushels at Brandon and 4.7 bushels at Ottawa.

ACKNOWLEDGMENTS

The author wishes to express his indebtedness to Mr. T. D. C. Hamilton Plant Products Division, Production Service, Dominion Department of Agriculture, Ottawa, for supplying the seed used in the experiments, and to the members of the Cereal Division staff for criticism and helpful suggestions in the preparation of this paper.

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EVIDENCE OF A SEX DIFFERENTIAL IN THE UTILIZATION OF SHELL CALCIUM BY THE CHICKEN EMBRYO[1]

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[Received for publication October 9, 1940]

The authors have reported recently (7) the existence of a sex difference in the weight of day-old chicks. They indicated that, when the body weight of the day-old chick was expressed as a percentage of the setting egg weight, and comparisons were confined to chicks of the same family, male chicks, on the whole, were heavier than their female sibs. This is not in accord with the conclusions reached by the earlier investigators who found no such sex difference. Their findings were reviewed in the first report (7).

Since the publication of our first paper (7), Asmundson (1) in a personal communication reports finding a similar sex difference in the weight of day-old poults. According to him this difference in the poults is perhaps more pronounced than in chickens.

To explain the results of the first study, it was suggested that the male embryo draws more calcium than the female from the shell and/or the male is more efficient in utilizing exogenous factors. The present data indicate that the male draws more mineral from the shell than does the female.

EXPERIMENTAL MATERIAL

As in the previous study, both Barred Plymouth Rocks and White Leghorns were used. The eggs were weighed to the nearest tenth of a gram within 24 hours of laying, and the specific gravity of each egg was obtained by the method described by Munro (6). The eggs were gathered daily over a period of 3 weeks before being set. In this way, some of the eggs were kept for as long as the full period of 3 weeks, while others were stored only overnight before setting. At the time of setting, all the eggs were again weighed, with the procedure repeated on the eighteenth day of incubation. All chicks were hatched in individual compartments provided with specially made paper cups placed at the bottom of each compartment to hold the shell. As soon as dry, the chicks were individually identified and weighed to the nearest tenth of a gram. The shell was air dried to constant weight and the individual samples weighed to the nearest milligram on a chainomatic balance. Care was taken to remove from the paper cup any debris, such as bits of dried yolk, faeces and particles of down, before weighing the shell.

RESULTS

Following the plan of analysis laid down in the first paper (7), the weight of the chick and of the shell which remained behind on hatching, were each expressed as a percentage of the setting egg weight. This

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effectively removed the influence of any weight differences between the individual eggs. Again the progeny of each dam was considered as the largest unit for analysis and all comparisons were made within dams. The mean chick and shell weights, expressed in each sex as percentages of the original setting weight, were then considered as paired observations suitable for analysis by "Student's" method. As there were two hatches in each breed, at first the comparisons were made within each hatch, and then the data were pooled in such a way that each hatch contributed equally to the final result. The data are summarized in Table 1.

TABLE 1.—COMPARISON OF THE SEXES WITH RESPECT TO CHICK WEIGHT AND TO RESIDUAL SHELL WEIGHT* WHEN EXPRESSED AS A PERCENTAGE OF SETTING EGG WEIGHT

Breed	No. of dams	Weight of shell*		Weight of chicks	
		Mean difference in favour of eggs producing females	*Z* value. Odds in brackets	Mean difference in favour of males	*Z* value. Odds in brackets
		%		%	
B.P. Rocks	25	0.12	0.32 (17.5 : 1)	0.50	0.24 (7.61 : 1)
White Leghorns	29	0.16	0.47 (69.9 : 1)	1.00	0.56 (237 : 1)

* Shell remaining after hatching.

It will be noted that in both breeds the male chicks were larger in proportion to the setting egg weight than their female sibs, This corroborates the results reported in our first paper. However, only in the case of Leghorns was the difference significant. It can also be seen that a greater proportion of non-utilized shell remained behind in the case of the females.

As was suggested previously (7), one plausible explanation of the sex difference in body weight of day-old chicks is the higher rate of utilization of shell calcium by the male embryo. If this is correct, then the male embryo would be expected to leave behind proportionately less shell than the female. The data in Table 1 show that this is actually what occurs, and it would appear that at least part of the relatively larger size of the male chick is due to a proportionately greater utilization of shell minerals.

Further evidence of the degree of interdependence of body size on shell mineral utilization can be secured by correlating the degree to which the males of each family exceed the females, with the relative excess of shell left by the females. When this is done for the 54 families in Table 1, the net correlation, with the effect of breeds removed by covariance, is 0.361, the necessary level for significance at the 1% point being 0.351. This means that, as the weight of the male chicks becomes increasingly greater than their sisters', when both are expressed as a percentage of the original egg weight, the relative weight of the shell left by the females increases over that left by their brothers. In other words we may now quite definitely state that the proportionately larger size of the male chick is due partly to a relatively greater utilization of shell minerals, most of which is, presumably, calcium. Jull (2) states that, during incubation, 75% of the calcium of the newly hatched chick comes from the shell.

There is one obvious criticism of the results presented thus far. It can be argued that the higher residual shell weight of the female chick may be due to an originally heavier shell. Olsson (9) found a high degree of correlation (0.982) between the specific gravity of the egg and the weight of its air-dried shell, when the latter is expressed as a percentage of the corresponding egg weight. His method is the most practical manner of measuring the percentage of shell in an egg while leaving it intact for incubation.

The specific gravity of all eggs in this experiment was recorded to the nearest third decimal place shortly after the egg was laid. When the specific gravities of the eggs giving rise to male and female chicks were compared in each family by the method of paired observations, no difference was found. It may be concluded, therefore, that eggs giving rise to female chicks do not possess heavier shells when fresh, than those producing male chicks.

As a matter of routine, the weight of the egg on the eighteenth day of incubation was obtained, in addition to the setting egg weight. With these further data on hand, it was considered advisable to determine whether or not the sex difference present in the weight of the day-old chicks already exists by the eighteenth day of incubation. For this purpose, the eighteenth day weight of the egg was expressed as a percentage of setting egg weight. The method of paired observations was again applied to the data to determine whether the two sexes from each family showed any difference in weight at this stage. This method of analysis was applied both to the data secured in the spring of 1940 and to the 1939 material used in the previous investigation (7). The analysis failed to show any difference.

TABLE 2.—COMPARISON OF THE SEXES WITH RESPECT TO CHICK WEIGHT PLUS RESIDUAL SHELL WEIGHT EXPRESSED AS A PERCENTAGE OF SETTING EGG WEIGHT

Breed	No. of dams	Mean difference in favour of males	*Z* value. Odds in brackets
		%	
B.P. Rocks	25	0.39	0.19 (4.94 : 1)
White Leghorns	29	0.68	0.37 (23.0 : 1)

It is obvious from the data presented in the first paper and the additional information reported here, that while there is a sex difference in the weight of the day-old chicks, the corresponding sex difference in the residual shell weights is, relatively speaking, much smaller. This latter difference was only 0.12% for Barred Rocks and 0.16% for White Leghorns. It does not seem, therefore, that this sex difference in the amount of residual shell wholly accounts for the final difference in the day-old weights of the sexes.

Romanoff (12) found that male chicks, on the average, have larger internal organs than females hatched from eggs of similar size. The only exception to this was the weight of the liver and the length of the caecum.

Lachat (4) found a slightly higher percentage ash in the bones of female chicks, whereas O'Neil (10) of this laboratory reports a slight difference in favour of males. In neither case was the difference statistically

significant. It would appear, therefore, that there is little or no difference in degree of calcification between the sexes at hatching time. However, it is generally recognized that the long bones of day-old males are slightly larger than those of females which, if true, means that the males possess a higher absolute amount of calcium. This agrees with our findings concerning the greater utilization of shell calcium by the male and with Romanoff's report regarding organ weights in the two sexes. To support heavier viscera, the male chick needs sturdier bones.

No data were obtained in the present investigation on the weights of the individual organs or long bones. Sufficient material has been secured, however, to answer the question postulated above, viz., whether the sex difference in the amount of shell, as obtained in this study, fully accounts for the heavier hatching weight of the male chick. Obviously, if it does, the corresponding shell and chick weights, when added together and expressed as a percentage of the setting egg weight, should be equal in the two sexes. This has been done and is summarized in Table 2. The results show that the male chick weights plus the residual shell weights still average heavier than the corresponding figures for the females. In the case of Leghorns, the difference exceeds the 5% error point. We may conclude, therefore, that the proportionately larger amount of shell utilized by the male chick during incubation does not account for all of the difference in the hatching weight of the sexes. The remainder must consist of muscle, viscera, etc.

DISCUSSION

Murray (8) found that the shell loses weight during incubation. According to Lippincott and Card (5), "The embryo seems to draw on the shell exclusively for its supply of calcium without making use of what is in the yolk until after hatching". The hypothesis, postulated in the previous report (7) that the male chick embryo draws more on the shell for its calcium supply than does the female has been substantiated in the present investigation. The available data show that the shell remaining from the egg which gives rise to a male chick is proportionately lighter than the shell of the egg from which the female chick hatches.

This tendency for the male chick to take more calcium out of the shell is particularly well indicated in the case of the Leghorn breed, although the same trend is apparent in the Barred Plymouth Rocks. Why the male embryo should draw more calcium from the shell than the female is not clear. Perhaps a higher metabolism in the male sex is responsible for this. Kagiama (3) found a higher oxidation rate in the male embryo beginning on the twelfth day of incubation. Plimmer and Lowndes (11) report a marked increase in the calcium intake of the growing embryo occurring about the twelfth day of incubation. This coincides in point of time with the increased metabolic rate of the male. When these findings are considered in conjunction with the authors' (7) previous report showing that male chicks are actually heavier than their female sibs and with Romanoff's conclusions concerning the greater organ weights of male chicks, it seems logical to conclude that during the later stages of incubation the male chick is characterized by a naturally greater rate of development. This would call for a greater utilization of shell calcium on the part of the male embryo.

SUMMARY

1. Data presented here corroborate results reported in the previous investigation that there is a sex difference in the weight of day-old male and female sibs. The difference is in favour of males.

2. The difference in weight of the two sexes is not apparent at the eighteenth day of incubation.

3. The male chick embryo draws more on the shell for its calcium supply than does the female chick. However, this greater utilization of shell calcium on the part of the male which, presumably, results in larger bones, does not account for all of the sex difference. The larger bones, probably, are accompanied by heavier muscles and viscera.

4. The proportion of shell in the new laid egg, as measured by specific gravity, is not related to the sex of the chick which hatches from it.

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EFFECT OF SOIL DROUGHT ON WHEAT PLANTS[1]

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[Received for publication July 4, 1940]

The growing of wheat in many parts of the world, including the Prairie Provinces of Western Canada, is carried out on land receiving limited rainfall. Soil moisture shortage often occurs in these areas, with the consequence of reduced yields or crops not fit for harvesting. Wheat, like all plants, requires considerable moisture to carry out its life processes. From the time the seed is sown into the soil until the later maturation stages, water plays a very important function. It is necessary for germination, it is necessary for cell development, it enters into the photosynthetic and enzymatic processes, it assists in the transportation of materials through the plant, and it acts as a buffer against extremes of high and low air temperatures. In the early stages of growth, water makes up about 90% of the composition of the wheat plant, and the water content remains high throughout its whole growing period. Moreover, the total water content is small in comparison with the amount which passes through the plant, as the greater proportion of the water absorbed from the soil is eliminated unchanged and dispersed into the atmosphere. In order to study some of the effects of scanty water supplies on the wheat plant, an investigation was carried out in the greenhouse to measure certain morphological and physiological differences between plants receiving ample water supplies and those receiving reduced water supplies. These investigations were carried out during the fall and winter months of 1937–38 at McGill University, and at Ottawa during the summer and fall months of 1938 and 1939.

EXPERIMENTAL METHODS

Definite quantities of soil of known moisture content were potted into 6-inch clay pots, and 5 seeds were sown to each pot. The resulting plants were allowed to grow under optimum conditions to the stage at which withdrawal of watering was commenced. For the controls, frequent watering with an amount each time to bring the soil moisture to 21% kept the plants supplied with ample water for growth and development at all times. Where more than one period of soil drought was given, the pots were watered to bring the soil moisture to 15% level, and then allowed to dry down. The pots were bunched into compact groups, and the position of each was altered frequently to bring about as uniform conditions as possible. Air temperatures rose to 80° to 90° F. or higher in the day, and dropped to as low as 50° F. at night. Humidities varied considerably throughout the tests. In other words, no attempt was made to control temperature, humidity or light throughout the experiments. However, artificial illumination was used to supplement daylight in the winter time by suspending a 250-watt light bulb over the plants from early morning to midnight. Variations from the above procedure are recorded in the text.

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Measurements on different parts of the plant constituted the structural studies.

For the cellular studies 3 main investigations were made, osmotic pressure, permeability of the cells to urea solutions, and desiccation of leaf sheath tissues. The mesophyll cells of the leaf sheath are more suitable than the leaf blade tissue for studies of this nature since the cells are more regular in shape and contain much less chlorophyll. The leaf sheath tissue of the 2nd node leaf was principally used for both normal and soil droughted plants. In the latter series the plants were usually allowed to develop normally beyond the 2nd node leaf sheath stage in order that this part of the plant would be comparable in morphology to that of the check plant.

The osmotic pressure was obtained by the plasmolytic method. Longitudinal sections of the leaf sheath were placed in solutions of $CaCl_2$ of known molar concentration and with the aid of neutral red dye the concentration of the solution which balanced the tension or is isotonic to the cell was obtained and translated into atmospheres of osmotic pressure. The validity of using the plasmolytic method for determining osmotic pressure has been discussed by Levitt and Scarth (6) and need not be repeated here.

For the permeability of the cells to urea it was first necessary to find out the osmotic pressure of the cells. With this figure an urea solution was used which had twice the osmotic pressure of the cells. When the tissues were placed in this solution the cells plasmolyzed to approximately $\frac{1}{2}$ their original volume and through time expanded back to it. The time required to effect this change was recorded as the permeability of the cells to urea. Levitt and Scarth (7) employed several substances for permeability tests and obtained satisfactory results with such polar non-electrolytes as urea and thiourea. The former substance when applied to wheat plant cells proved very satisfactory in these tests and was used exclusively.

Iljin (4) described a method for studying the desiccating resistance of cells of different plants, and this method was adapted for studying the desiccating resistance of cells of the wheat plant. Chambers were made up from glass rings about 25 mm. in diameter by 10 mm. high and these were mounted on a series of glass slides and attached by means of Dekotinsky cement. Cover slips were made from thin microscopic slides and these were used for superimposing the sectional tissue over different concentrations of the desiccating agent. Longitudinal sections of the leaf sheath tissue about 2 to 3 mm. wide by 8 to 10 mm. long were cut by drawing a sharp razor down the leaf sheath just below the upper epidermis (epidermis nearest the stem). The leaf sheath tissue was placed with the cut side up on the moistened cover slip and 4 pieces were used with each chamber. The moisture was blotted off the sections and the cover slip was inverted over the ring and sealed with stop cock grease. Stock solutions containing different weight molar concentrations of NaCl were made up to serve as the desiccating agent. In each chamber was placed the desired concentration of NaCl to a depth of about 5 mm. which allowed the tissue to be brought close to the surface of the solution. The necessity of having the sections close to the surface is important in order to obtain the relative humidities desired.

When a series was made up the chambers were placed in a box kept in a basement room held at close to 20° C. After 48 hours the clover slips were removed from each chamber and the NaCl solutions were replaced with water. The tissue on the cover slips at this stage had shrunken considerably, especially that which was superimposed over the higher concentrations of the NaCl solutions. Any sections which had dropped into the solutions were discarded. After 24 hours the sections had regained their original size by taking up the moisture from the humidified air over the water in the chambers and were ready for testing the viability of the cells.

Solutions of about 0.1 molar concentration of a combination of NaCl and $CaCl_2$ (9 : 1) and containing 50 p.p.m. of neutral red dye (Dr. G. Grübler & Co. vital for injection) were prepared and the sections were placed in these solutions and allowed to take up the dye. The viability of the cells was then observed under the microscope either directly or by first plasmolyzing them in $CaCl_2$ solutions. This latter procedure was adopted when there was little accumulation of the dye in the living cells. For the dead cells only the outline of the cell walls and faint outlines of the disintegrated protoplasm could be seen. It was not difficult to ascertain the living cells as they were usually well stained and would plasmolyze and deplasmolyze in hypertonic and hypotonic salt solutions. The appearance of the chloroplasts was an additional guide to the viability of the cells.

In recording the behaviour of the cells in the desiccating tests, symbols instead of actual counts of living cells were used. The designations are as follows:—

++++ — A large mass of living cells.
+++ — About 75% of living cells.
++ — About 50% of living cells.
+ — About 25% of living cells.

Lesser numbers of living cells were recorded as several or few while 0 was given to 100% destruction. In making the observations due allowance was given to parts of the tissue which were injured when sectioning.

The procedure for the desiccating studies is given rather fully since the technique is not simple but requires considerable attention to detail. It does, however, offer a means for comparing directly the ability of the protoplasm to stand the stresses set up by desiccation. The nature of these stresses has been followed by Iljin (5) in observing the behaviour of red cabbage cells through desiccating influences. As will be seen later the method served to show the greater ability of cells of wheat plants "hardened" by soil drought and by low temperatures over those from normally grown plants to withstand these forces.

PLANT MEASUREMENTS

The main feature of the investigation was directed toward cell studies, but certain plant measurements were made to determine some of the gross effects of scanty soil water supplies on the wheat plant.

A series of tests was conducted on plants of 2 varieties of wheat over a period of 22 days. The plants at the beginning of the experiment had developed beyond the 2nd node leaf stage. One set of pots of each variety

TABLE 1.—PARTIAL DESCRIPTION ON SINGLE PLANT BASIS FOR CHECK AND SOIL DROUGHTED SERIES

Drought period	Variety	No. of plants	*Height	Length of leaf blades		Length of sheath		Distance between nodes	
				2nd	4th	2nd	4th	1st & 2nd	3rd & 4th
days			m.m.	m.m.	m.m.	m.m.	m.m.	m.m.	m.m.
9	Marquis (check)	5	412	264	300	111	155	59	83
	Marquis (droughted)	5	273	275	189	106	97	44	47
	Canus (check)	5	433	253	288	102	133	51	86
	Canus (droughted)	5	271	264	199	103	77	47	56
22	Marquis (check)	5	462	273	289	112	158	49	122
	Marquis (droughted)	5	311	262	182	108	97	41	61
	Canus (check)	5	490	261	363	108	136	52	98
	Canus (droughted)	5	370	272	215	108	96	48	72

* Height taken from crown to ligule of top culm leaf.

TABLE 2.—NUMBER AND AVERAGE DISTANCE APART IN MILLIMETRES FOR VEINS IN CROSS-SECTIONS OF FOURTH NODE LEAF BLADE OF CHECK AND DROUGHTED PLANTS

Variety	Check plants			Droughted plants		
	Plant number	Number of veins	Average distance apart	Plant number	Number of veins	Average distance apart
Marquis	1	30	.3495	4	32	.2399
	2	32	.3146	5	34	.2574
	3	38	.3491	6	33	.2737
	Mean	33	.3383	Mean	33	.2572
Canus	7	35	.3364	10	34	.2593
	8	38	.3257	11	33	.2385
	9	48	.3013	12	36	.2605
	Mean	40	.3191	Mean	34	.2531

was watered to bring the soil moisture to 21% every 48 hours, while a second set was watered to a calculated 15% of soil water 4 times throughout the period.

In Table 1, measurements on height, length of leaf blades, length of sheath and distance between nodes, are given for 9- and 22-day periods. It will, therefore, be seen that the check plants grew taller and produced longer leaf blades and sheaths on the 4th node and showed greater elongation between the 3rd and 4th node.

The number and average distance apart of the veins, and counts of stomata on upper and lower 4th leaf blades were determined on checks and soil droughted plants. As will be seen in Table 2, the number of veins was not less, but the distance apart was definitely less for the droughted plants. In Table 3, it will be noted that the number of stomata per unit

TABLE 3.—STOMATA COUNTS PER 1.1033 MM.² FOR THE 4TH NODE LEAF BLADE OF CHECK AND DROUGHTED PLANTS

Variety	Plant number	Check plants		Plant number	Droughted plants	
		Upper	Lower		Upper	Lower
Marquis	1	46.2	29.6	4	79.8	67.7
	2	49.1	38.3	5	98.9	52.4
	3	54.6	37.4	6	92.5	72.8
	Mean	50.0	34.4	Mean	90.4	64.3
Canus	7	44.6	32.3	10	96.5	61.8
	8	56.4	41.3	11	119.5	64.5
	9	54.3	42.3	12	80.9	61.6
	Mean	51.8	38.6	Mean	99.0	64.3

NOTE—Ten counts were made on each upper and lower leaf blade respectively.

area for the 4th node leaf blade was nearly twice as great in the soil droughted plants. While measurements were not made on the size of stomata, they were much smaller for the 4th node leaves on these plants.

The dry weights of shoots and roots together with the ratio of roots to shoots were obtained for the soil droughted and check plants. The check plants having developed to a greater extent than the soil droughted plants, produced dry weights nearly twice as great. However, the ratio of shoots to roots was not materially changed, thus indicating that growth was restricted to approximately the same extent in the roots as in the shoots under reduced soil water supplies.

The total loss of water per pot was determined over the 22-day period for checks and soil droughted plants. The loss of water was in the order of about 3 times the quantity for the check pots over the soil droughted pots. By watering pots without plants in the same manner as those with plants, it was calculated that the transpiration loss in grams per plant was 5 to more than 6 times greater for the check plants. Over a 12-day period the average transpiration loss per plant for the checks amounted to 100 grams, and for the soil droughted plants 17 grams.

The above structural studies indicate quite clearly that the growth of the parts of the plant developed under scanty soil water supplies is much more restricted than when ample water is available to the plant. This restricted growth is largely attributed to the production of smaller cells in the tissue during this period.

CELLULAR STUDIES

Osmotic pressure, permeability of the cells to urea solutions, and desiccation measurements were applied to several series of wheat plants.

Experimental Results of One Period of Soil Drought

In the first experiment, plants of 2 varieties were allowed to grow under ample soil water supplies to the 4th node leaf stage, and then watering was discontinued. Every 3 days one plant from each of 8 pots was removed at each date for cellular studies, and a similar set was used for plant moisture

studies. In Table 4, the osmotic pressure, permeability to urea, plant moisture, water per 100 grams of dry matter (by calculation) and soil moisture are given. The osmotic pressure permeabilities to urea were taken on the mesophyll cells of the 2nd node leaf sheath. It is here noted that a marked increase in osmotic pressure was obtained almost immediately, and 3 days later the rate of permeability to urea had increased

TABLE 4.—OSMOTIC PRESSURE, PERMEABILITY TO UREA, PLANT MOISTURE, WATER PER 100 GRAMS OF DRY MATTER AND SOIL MOISTURE FOR 2 VARIETIES OF WHEAT

Date	Variety	O.P.	Permeability to urea	Plant moisture	Water per 100 gm. dry matter	Soil moisture
		At.	minutes	%	gms.	%
Jan. 10	Marquis	16.1	164	85.7	599	19.8
	Milturum	16.1	182	86.1	619	19.9
Jan. 13	Marquis	21.9	182	85.3	580	12.1
	Milturum	21.9	170	84.6	549	14.8
Jan. 16	Marquis	19.7	70	82.6	475	12.9
	Milturum	19.7	64	83.9	521	12.1
Jan. 19	Marquis	21.2	71	81.2	432	10.7
	Milturum	22.6	69	80.2	405	10.8
Jan. 22	Marquis	23.9	56	81.7	446	9.7
	Milturum	23.2	57	80.2	405	9.0
*Jan. 25	Marquis	16.1	148	84.2	533	19.7
	Milturum	16.1	137	85.0	567	20.8

* Checks.

TABLE 5.—RESISTANCE TO DESICCATION OF SECTIONS FROM SECOND NODE LEAF-SHEATH TISSUE IN CHAMBERS AT DIFFERENT RELATIVE HUMIDITIES INDUCED BY SOLUTIONS OF DIFFERENT MOLAR CONCENTRATIONS OF NaCl DURING A 48-HOUR PERIOD

—	—	NaCl concentrations in gram molecules per litre of solution					
		0.8	1.0	1.2	1.4	1.6	1.8
Jan. 10	Marquis	+++	+	0	0	0	0
	Milturum	+++	+	0	0	0	0
Jan. 13	Marquis	++++	++	Several	0	0	0
	Milturum	++++	+++	+	0	0	0
Jan. 16	Marquis	+++	+++	++	Several	Few	0
	Milturum	+	+++	+	Few	+	Few
Jan. 19	Marquis	++	+++	++	++	++	+
	Milturum	+	++	++	++	+	Few
Jan. 22	Marquis	++++	+++	+++	+++	Several	+
	Milturum	++	+	+	+	Several	Several
*Jan. 25	Marquis	++	+	0	0	0	0
	Milturum	+	Few	Several	0	Few	Few

* Checks.

TABLE 6.—RESISTANCE TO DESICCATION OF CELLS OF SECTIONS FROM SECOND AND THIRD NODE LEAF SHEATH TISSUE

Date	Variety	Treatment	Mols. of CaCl per litre of solution									
			0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6
Mar. 27	Marquis	Check	++++	++++	++	0						
	Marquis	Droughted		++++		++++		++	++++	++		
	Canus	Check	++++	+++	+++	++						
	Canus	Droughted		+++		+++		+++	++	++		
April 10	Marquis	Droughted		+++					++		+	+
	Marquis	Droughted*		+++					+++		++	++
	Canus	Droughted		++					++		+	+
	Canus	Droughted*		+++					+++		+++	++
April 14	Marquis	Check		+++			0		0			0
	Marquis	Check*		++			0		0			0
	Canus	Check		++			+		0			0
	Canus	Check*		++			0		0			0

* Sections from third node leaf sheath.

almost threefold. In the drying down of the soil, the cells increased in the amount of osmotically active substances, likely sugars, and a change had taken place in the protoplasm or the plasma membrane at least as indicated by the increase in the permeability to urea. The plant moisture obtained by drying all of the plant except the roots and any dead material in an air oven held at 100° C. for 24 hours, progressively decreased with the drying down of the soil.

The results for the desiccation studies presented in Table 5 indicate that the cells of the leaf sheath tissue under normal watering conditions were unable to withstand the desiccating forces of NaCl solutions beyond 1 molar concentration, while those of the plants under soil droughted conditions withstood the desiccating forces of NaCl solutions up to 1.6 molar concentrations with some cells remaining alive over 1.8 molar NaCl for a period of 48 hours. We would, therefore, seem justified in concluding that a "hardening" of the plant cells took place under soil drought; this was shown by a greater increase in the resistance of the cells to desiccation and which was accompanied by an increase in osmotically active substances in the cells and a change in the permeability of the cells.

Experimental Results for Several Periods of Soil Drought

A second series of tests was conducted on plants of 2 varieties where several periods of soil drought were introduced. In this experiment osmotic pressure values as high as 29 atmospheres were obtained for the mesophyll cells of the leaf sheath tissue for plants grown in pots under reduced water supplies. The urea permeabilities were in about the same ratio for normal watering as for one soil drought period reported above.

The ability of the mesophyll cells in sections of leaf sheath tissue to withstand desiccation when superimposed over solutions of NaCl solutions will be seen in Table 6 to be much greater for the droughted plants than for the check plants. Sections from 2nd and 3rd node leaf sheath tissue taken on April 10 from droughted plants contained a large number of

TABLE 7.—OSMOTIC VALUE AND RELATIVE HUMIDITY OF NaCl SOLUTIONS

Mols. of NaCl per litre of solution	Osmotic value in atmospheres at 20° C.	Relative humidity in %
.1	4.33	99.68
.4	16 57	98.77
.6	25.66	98.10
.8	34.24	97.47
.9	38.56	97.14
1.0	43.2	96.8
2.0	96.4	93.0
3.0	166.0	88.3
4.0	249.0	83.0
5.0	323.0	78.4
Saturated	369.0	75.8

cells which remained alive even when superimposed over NaCl solutions of 2.6 molar concentrations for a period of 48 hours. If we look at Table 7 it will be noted that this corresponds to an osmotic pull of considerably

TABLE 8.—RESISTANCE OF DESICCATION OF MESOPHYLL CELLS OF LEAF SHEATH TISSUE OF WHEAT PLANTS "HARDENED" BY SOIL DROUGHT*

Plant stage	Variety	Molar concentration of NaCl in desiccating chambers				
		1.0	1.5	2.0	2.5	3.0
July 5						
Head in boot	Marquis	+++	0	0	0	0
Head in boot	Reliance	+++	+++	+	0	0
Head in boot	Caesium O111	+++	+	Few	0	0
Head in boot	Cae. × Ma.	++++	++++	0	0	0
July 7						
Head in boot	Milturum 0277	++++	++	+	0	0
Head emerging	Thatcher	+++	++++	0	0	0
Head in boot	Cheyenne*	+++	+++	++++	0	0
July 10						
Head emerged	Marquis	+++	++	+	0	0
Head emerged	Caesium O111	++++	+++	0	0	0
Flowering	Regent	++++	++++	Several	0	0
July 17						
Kernels advanced	Marquis	—	+	++	+	Odd
Kernels formed	Tenmarq†	++++	+++	Odd	0	0
Flowering	Kharkov 22 M.C.†	+++	++	++	+++	0
Flowering	Minturki†	+++	+++	++	—	Odd

* Seeds sown in bins containing 1 ton of soil to depth of 14" in greenhouse May 19/38.
† Winter wheat varieties. Plants grown from vernalized seed.

more than 100 atmospheres of osmotic pressure. Cells from plants grown under normal water supply did not survive over NaCl solutions corresponding to osmotic pulls beyond 60 atmospheres, and in most cases did not survive beyond osmotic pressure pulls corresponding to 43 atmospheres.

Experiments Conducted in Summer of 1938

Since the desiccation studies appeared to offer the best promise for showing the extent to which cells of wheat plants "hardened" by soil drought further tests were made during the summer of 1938. In pot experiments the drying down of the soil is fairly rapid and it was thought that if the plants were grown in a large mass of soil a longer period would be afforded the wheat plant in which to adjust itself. Consequently, a bin containing about 1 ton of soil to the depth of 14 inches was set up in the greenhouse. Curtains were provided to shade the house on excessively hot days. Seed from both spring and vernalized winter wheat varieties was planted on May 19 in soil containing 17.5% moisture. The vernalization was accomplished by the method described by Gfeller and Fraser (1). Forty pounds of water was sprayed on to the soil 3 times with the last addition of water being made on June 14. By June 27 the soil moisture had dropped to 8.4% and by July 6 to 6.0%. Desiccating studies were made on a number of plants at 4 different dates.

In Table 8, the stage of growth for the plants of the different varieties studied as well as the results of the desiccation tests are recorded. For the tests made on July 5 the varieties Reliance and Caes. × Marquis and Caesium appeared to be superior to Marquis. On July 7 comparisons made between

Milturum 0277, Thatcher, and Cheyenne, the last named variety showed the highest desiccating values. The plants of Cheyenne, a winter hardy winter wheat, were grown from vernalized seed. For the tests made on July 10 little differences were obtained between Marquis, Caesium and Regent. On July 17 plants of 3 winter wheat varieties and 1 spring wheat were compared. It will be observed that the plants of Marquis were in the advanced stages of maturity and good resistance of the cells to desiccation was obtained. Of the winter wheat varieties, Kharkov 22 M.C. and Minturki in the flowering stage gave high resistance values to desiccation.

Plants grown in pots were also studied and comparisons were made between 3 varieties in advanced stages of plant development. For these tests as will be seen in Table 9, Canus and Milturum showed up to better advantage than did Marquis.

TABLE 9.—RESISTANCE TO DESICCATION OF MESOPHYLL CELLS OF LEAF SHEATH TISSUE OF PLANTS "HARDENED" BY SOIL DROUGHT*

Plant stage	Variety	Molar concentration of NaCl in desiccating chambers				
		1.0	1.5	2.0	2.5	3.0
July 10/38						
Kernels formed	Marquis	++++	++	Few	0	0
Kernels almost formed	Milturum	+++	++	+	Several	Several
Kernels formed	Canus	+++	++++	+++	+	0

* Seeds sown on May 13/38 into glazed pots and plants given 3 droughting periods. Tissue of 3rd node leaf sheath used.

Experiments Conducted in Summer of 1939

The experiments conducted in the spring and summer of 1938 suggested that possibly cells of plants of winter hardy winter wheat varieties had a greater ability to resist desiccation than those of the spring varieties. Consequently a number of 6″ × 6″ glazed pots were planted on May 3, 1939 to seed of spring wheat varieties and to vernalized seed of winter wheat varieties. In addition, plants of winter wheat varieties and clones from 2 grasses were taken from the field and transplanted into pots at the same time.

In Table 10, the data of the desiccating tests for 5 different dates are given. It will be observed that cells of the leaf sheath tissue of Kharkov 22 M.C. (transplanted), Marquis, and Caesium from plants grown under ample water supply had much less resistance to desiccation than for those plants from pots deprived of water. Moreover, it is evident that the cells of the winter wheat plants and of the 2 grasses, *Agropyron cristatum* and *Agropyron elongatum*, showed higher resistance to desiccation when deprived of water than those of the spring wheat varieties. Mesophyll cells of leaf sheath tissue of Kharkov 22 M.C. grown from vernalized seed even resisted desiccation from 3 molar solutions of NaCl, which corresponds to an osmotic value of 166 atmospheres and a relative humidity of about 88%.

Experiments Conducted in the Autumn of 1939

In the foregoing experiments it was shown that mesophyll cells of leaf sheath tissue taken from plants of wheat varieties receiving ample water

TABLE 10—RESISTANCE TO DESICCATION OF MESOPHYLL CELLS OF LEAF SHEATH TISSUE OF PLANTS "HARDENED" BY SOIL DROUGHT*

Plant stage	Variety	Treatment	Molar concentration of NaCl in desiccating chambers				
			1.0	1.5	2.0	2.5	3.0
June 12	Transplanted	No water added since June 3					
Flag leaf	Kharkov 22 M.C.†	Check	Odd	0	0	0	0
Flag leaf	Kharkov 22 M.C.†	Droughted	++	+++	++	0	0
June 16	Seeded	No water added since June 3					
Flag leaf	Marquis	Check	0	0	0	0	—
Flag leaf	Marquis	Droughted	++	++	Few	0	—
Flag leaf	Caesium 0111	Check	Few	0	0	0	—
Flag leaf	Caesium 0111	Droughted	Few	Few	0	0	—
June 19	Transplanted	No water added since June 3					
Flag leaf	Kharkov 22 M.C.†	Droughted	+++	++++	++++	++++	—
Head in boot	Quivira	Droughted	++++	+++	+++	+++	—
Culm	*Ag. cristatum*	Droughted	+++	+++	++	+	—
Culm	*Ag. elongatum*	Droughted	+++	++	++	0	—
June 22	Seeded	No water added singe June 10					
Headed	Marquis	Droughted	++++	+++	Odd	0	—
Headed	Caesium 0111	Droughted	++++	+++	0	0	—
July 3	Seeded	Vernalized seed of Kharkov 22 M.C. Quivira					
2nd Node	Kharkov 22 M.C.†	Droughted	—	—	++	++	++
3rd Node	Quivira†	Droughted	—	—	+++	+	0
3rd Node	Golden Ball	Droughted	—	—	0	0	0

* Seeds sown or plants transplanted into 6" × 6" glazed pots May 3/39.
† Winter wheat varieties

supplies had only a moderate resistance to desiccation and that this resistance was much greater for plants receiving limited water supplies. The experiments also indicated that the cell resistance of winter wheat plants was greater than those of spring wheat. This led to comparisons between plants of spring and winter wheat varieties grown in the field at Ottawa, Ontario, in the fall months under the influence of low temperatures. The seed was sown in rows on September 13 and the plants studied at 4 different dates in November. On November 8, a departure from the regular procedure was made in that the desiccated tissue was not allowed to take up water before its immersion in a hypotonic solution of NaCl and $CaCl_2$ (9 : 1) containing the neutral red dye. This is a more drastic treatment than the usual procedure, but the results obtained as shown in Table 11, are quite interesting. Cells of Kharkov 22 M.C., the most winter hardy wheat, demonstrated the best ability to resist desiccation and subsequent treatment. Cells of Marquis spring wheat, which has little resistance to low temperatures, demonstrated the least resistance. For the other dates the regular procedure was employed. Turning to the comparisons made for November 30 it is quite evident that Kharkov 22 M.C. showed the greatest desiccating resistance and Marquis and Thatcher the least. Moreover, it would appear that the varieties in these tests arranged themselves

TABLE 11.—RESISTANCE TO DESICCATION OF MESOPHYLL CELLS OF LEAF SHEATH TISSUE OF WHEAT PLANTS "HARDENED" BY LOW TEMPERATURES*

Variety	Molar concentration of NaCl in desiccating chambers							
	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Nov. 8—Sections desiccated for 48 hours and then removed from chambers and plunged into hypotonic solutions of 9 NaCl + 1 $CaCl_2$								
Marquis	++	Several	Several	Few	0	0	—	—
Dawsons G. Chaff†	++	++	+	+	Few	Several	—	—
Kharkov 22 M.C.	+	++	+	+	++	++		
Nov. 13—Sections desiccated for 48 hours and then allowed to take up water slowly								
Marquis	+++	+++	+++	+	Few	0	—	—
Dawsons G. Chaff†	++++	++++	++++	++++	++++	++++		
Kharkov 22 M.C.†	++++	++++	++++	++++	++++	++++		
Nov. 17—Same procedure as for Nov. 13								
Dawsons G. Chaff†	—	—	—	—	++++	++++	Few	0
Kharkov 22 M.C.†	—	—	—	—	++++	++++	++++	Few
Nov. 30—Same procedure as for Nov. 13								
Marquis	—	—	++	Few	+	0	—	—
Thatcher	—	—	++	Few	+	0	—	—
Canus	—	—	+++	+++	++	++	—	—
Quivira†	—	—	—	—	++++	+++	++	Few
Kharkov 22 M.C.†	—	—	—	—	+++	+++	+++	+

* Seeds sown in field at Ottawa Sept. 13/39 and plants in the Rosette stage taken from field in November.
† Winter wheat varieties.

in the order of their known relative low temperature resistance. Kharkov 22 M.C. is the most winter hardy of the winter wheat varieties while the spring wheat varieties have little resistance to the usual Canadian winters. For the particular series studied the spring survival was noted for the remaining plants in the field. The 4 winter wheat varieties showed 100% survival with little or no checking back. The plants of Marquis were all killed while those of Canus and Thatcher were either killed outright or checked severely. Canus showed a slight superiority over Thatcher in the vigour of the few plants remaining but none of the plants of the 2 spring wheat varieties could be compared in vigour to those of the winter wheat varieties.

DISCUSSION AND SUMMARY

The wheat plant adjusts itself to soil water supplies and when these are limited during the stages of cell development of any part of the plant smaller cells are produced. The height of the culm, the size of the leaves, the distance between the veins and the distance apart and size of stomatal openings are all affected when they are developing under limited water supplies. This reduction of growth should be a protection to the plant in that it reduces the succulency of the cells and presents a smaller surface for moisture losses.

In the greenhouse experiments the plant moisture percentage was lowered and the transpiration was greatly reduced by soil drought. The marked reduction in transpiration cannot all be attributed to the smaller surface as transpiration was accelerated when water was added to the soil. The greatest factor for slowing up transpiration would appear to be the inability of the plant to obtain water from the soil. It is also assumed that the plant adjusted itself physiologically to excessive water loss.

There is a marked rise in the osmotic pressure of the cells by soil droughting. Vasiliev (9) working with wheat plants observed that as the water supply decreases and the water deficit in the tissues increases, the complex carbohydrates break up into simpler ones. An increase in the water content of the plants brings about the reverse process resulting in a decrease of soluble carbohydrates. Newton and Martin (6) studying a number of crop plants found that osmotic pressure varied according to scarcity of water but concluded that this was not as good an index of drought resistance as their bound water measurements.

The marked increase obtained for the permeability of the cells to urea would support the theory that the protoplasm or plasma membrane at least had become more hydrophyllic. Similar permeability changes were found by Levitt and Scarth (5) to occur in plant cells "hardened" by low temperatures. They postulated the theory that this would assist in preventing damage to the cells from quick and slow freezing by allowing a more rapid passage of water out of the cell. It might be argued that in dehydration due to desiccation greater stresses would be set up by the more rapid flow of water out of the cell and hasten its collapse. This might be offset by the argument that an increase in the permeability of the cells would assist in the more rapid passage of water from cell to cell and thus protect those furthest remote from the veins.

The increase in permeability and in osmotic pressure accompanying soil drought are not in themselves sufficient to explain the marked increase in the resistance of cells to desiccation. Iljin (2) states that there is no doubt that the protoplasm undergoes purely mechanical injuries by drying and becoming wet and in this way its structure is destroyed. Siminovitch and Scarth (8) have noted that in extra-cellular freezing the resulting dehydration causes the cells to collapse, the opposite walls coming together and squeezing the contents to the periphery. A somewhat similar situation exists from dehydration due to desiccation. In the experiments reported in Table II the data demonstrated the greater susceptibility to injury of the desiccated cells of wheat plants hardened by low temperatures when they were not allowed to imbibe the water slowly. Resistance of the cells against injury by dessication would, therefore, appear to be associated with changes in the protoplasm which make it less susceptible to the mechanical stresses set up by excessive water loss.

The wheat plant is not regarded as highly drought resistant in that it cannot survive long periods of drought. Neither can it be considered to be resistant to very low temperatures even among our most winter hardy varieties. It does, however, tend to adjust itself advantageously in order that it may better withstand conditions of water scarcity and of low temperatures. In the desiccating studies, varietal differences were indicated for plants "hardened" both by soil drought and by low temperatures and in these tests the varieties arranged themselves in a similar manner by both "hardening" methods. The most outstanding variety was Kharkov 22 M.C., which is considered the most winter hardy winter wheat grown in Canada. Platt (7) in his studies on frost reaction on seedlings of wheat varieties pointed out that the majority of the relatively high resistant spring wheat varieties are selections from crosses between spring and winter types. The writer believes that crosses between spring and winter hardy winter wheat varieties might yield better drought resistant spring wheat varieties.

There remains the problem of selecting for drought resistance. Owing to its complexity a simple quantitative method may not cover all of the factors involved. Moreover, difficulties arise in applying such a technique to large segregating populations. Desiccating tests would not be feasible for such material but may be of service in the selection of parental varieties or in obtaining information on promising selections. There is the difficulty of control in the "hardening" of the plants by soil drought and it would appear to be useless to test "unhardened" plants. The final test for any wheat variety for areas subjected to limited moisture supply will be found in whether it has the ability to yield adequate returns under relatively dry conditions over a period of years.

ACKNOWLEDGMENT

The writer wishes to thank Dr. G. W. Scarth, Macdonald Professor of Botany, McGill University, Montreal, P.Q. for direction and advice in carrying out the experiments reported in this paper, and Dr. L. H. Newman, Dominion Cerealist, for his encouragement.

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COMPARISON OF THE WHOLE BLOOD AND TUBE AGGLUTINATION TESTS FOR PULLORUM DISEASE

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[Received for publication November 18, 1940]

In 1913, Jones (17) demonstrated that the macroscopic agglutination test was of great value in detecting carriers of *Salmonella pullorum*. His results were substantiated by Gage, Paige and Hylan (12) in 1914.

Rettger, Kirkpatrick and Jones (20) in 1915 issued their fifth report on bacillary white diarrhoea, in which they summarized their work on the agglutination test. They reported 10.24% positive reactors in 13,831 hens, and 2.9% positive reactors in 786 males. These were in 107 flocks. Retests on 13 flocks, after the removal of reactors, gave widely varying results. No reactors were found on the second test in 4 flocks, while in 9 flocks the infection varied from 0.6 to 25.7%. In each case, the percentage reaction was less on the second than on the first test. The same investigators (21) in 1916 issued their sixth report in which they state that a single agglutination test and elimination of reactors is not a guarantee that the flock has been entirely rid of ovarian infection. They considered this was due to the fact that certain birds have been infected for too short a time for the production of sufficient agglutinins to react to the test.

Gage and Paige (13) in 1915 recommended the use of the agglutination test for the eradication of bacillary white diarrhoea. They used a polyvalent antigen containing 6 strains of organisms obtained from various sources. Birds showing a positive agglutination in serum dilutions of 1-100, were considered reactors.

Ward and Gallagher (28) in 1917 reported on the value of the intradermal test for carriers of *S. pullorum*, emphasizing the importance of reading the test at a certain time.

Scherago and Benson (25) in 1919 reported their results from use of the intradermal test, and concluded that the test is so inconsistent that it is useless as a diagnostic agent for *S. pullorum*. They further reported that the introduction of the intradermal fluid caused at least 85% of the birds tested to react to the agglutination test regardless of their reactions to previous tests.

Subsequently, many investigators reported satisfactory progress in dealing with *S. pullorum* infections by using the macroscopic agglutination test.

Bushnell *et al* (9) in 1926 pointed out that while the macroscopic agglutination test (commonly called the tube agglutination test) was used widely, the technique of applying the test was not standardized, there being 16 different methods in 28 laboratories.

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In 1926, Huddleson and Carlson (16) reported on a plate method test for Bang's Disease, which was the same in principle as the slide test for Bang's disease suggested by Gwatkin (15) in 1922. The adaptation of this method for pullorum disease was reported by Runnels, Coon, Farley and Thorpe (22) in 1927.

In 1929, Bunyea, Hall and Dorset (7) reported on a test that could be applied in the poultry house, doing away with the expensive laboratory equipment and the collection of blood samples required for the tube agglutination and the rapid serum plate tests. However, their method had several disadvantages, because it required a live antigen; hence its handling was fraught with danger and it deteriorated rapidly.

In 1931, Schaffer, McDonald, Hall and Bunyea (24) published a modification of this test. It consisted of the preservation and killing of the bacteria with formalin, the addition of a dye to bring out the reactions more clearly, the use of a dropper for antigen and a loop to deliver a standardized amount of blood on a glass plate marked off in squares.

At the same time Coborn and Stafseth (10) announced a similar modification. The antigen was preserved with phenol, and dye was used to facilitate reading. They used standardized droppers to measure antigen and blood, and white porcelain was used instead of a glass plate.

Bleecker (6) in 1931 compared results of diagnoses on 2,159 birds by both the tube and whole blood tests. His diagnoses were in 91.15% agreement. Most of his discrepancies occurrred in the first flocks tested: at the close of his work he obtained almost identical diagnoses by both tests.

Biely and Roach (3) in 1932 reported on comparisons between the diagnoses made by the whole blood and rapid serum tests. They obtained an agreement of 98.7% with 2,932 diagnoses by each test. They used porcelain spot plates in place of glass and introduced glass covers to exclude dust and prevent evaporation. They also demonstrated the need of training and skill in reading reactions.

A second experiment by Biely and Roach (4) in 1932 compared diagnoses made by the tube, rapid serum and whole blood tests on a small group of birds, four tests being made at monthly intervals. The agreement obtained and the constancy of diagnosis by each method showed that all three tests were reasonably comparable and constant in diagnosis.

Bunyea and Hall (8) in 1932 reported on the examination of 206 birds by the stained-antigen, whole blood method and the tube agglutination test. There was an agreement of 91% between the two tests. The birds were killed for examination, and 114 of the 143 tube test reactors (79.7%) yielded *S. pullorum*, while this organism was recovered from 112 of 135 whole blood reactors (83%).

Durant (11) in 1932 tested a flock of 259 birds by the whole blood, plate serum and tube tests. The positive birds were purchased for bacteriological examination and, on the results of this, the tube test was placed first and the whole blood test last.

Graham, Thorpe and Torrey (14) in 1933 reported that in their experience a high degree of correlation had been obtained between the tube, rapid serum and whole blood agglutination tests in flocks subjected to repeated testing. They found that the whole blood agglutination test, in properly trained hands, constituted the simplest and most practical procedure for the diagnosis of pullorum disease in adult birds.

Schaffer and Bunyea (23) in 1933 reported that in one State, 190,000 birds were tested by the whole blood and tube agglutination tests, with an agreement of 98.8%.

Biely and Roach (5) in 1934 tested 4,429 birds by the whole blood test and sent blood samples to a commercial laboratory. The coefficient of agreement, according to Yule's method, was 0.998, which is very high. Blood samples from birds on which there were differences were sent to a second laboratory for confirmation. The original whole blood test and the rapid serum and tube tests at the second laboratory were in closer agreement than were the diagnoses by both laboratories. Discrepancies between the diagnoses by two laboratories were also reported by these authors, who secured closer agreement with the whole blood and rapid serum tests than did two laboratories both using the rapid serum test.

In 1935, Lubbehusen and Beach (18) did not consider that the whole blood stained antigen test should be exclusively used in a program of pullorum disease eradication. They stated that by retesting at short intervals with the whole blood test, the pullorum infected birds in a flock may be reduced to 1% or less, thus preparing it for accreditation by the tube test.

In 1940, laboratory workers in Massachusetts (1), reporting on twenty years' pullorum eradication work, using the tube agglutination test, showed that whereas there were 12.5% positive reactors out of 24,718 birds in 1920–21, there were 0.51% reactors out of 573,000 birds in 1939–40. The average reactors (positive), were less than 1% for all years subsequent to 1931. The average positive reactions for 1937–38, 1938–39, and 1939–40, were 0.17%, 0.34%, and 0.51%, respectively. These workers state that fowl typhoid carriers were detected in routine testing with pullorum antigen, and that fowl typhoid was eliminated by retesting at intervals of 2 to 4 weeks.

The importance of testing all of the birds to be used for breeding rather than a portion of the flock, is shown by McLellan (19) in 1939. In one set of experiments, a 100% test found infection in 16 out of 84 flocks. A 100% bird test of these flocks, found infection in 8 flocks, while a 10% test located four infected flocks. In a second test, a 100% test found infection in 14 flocks out of 73, while a 10% test found infection in 9 flocks only.

Results of the use of the whole blood test are reported by Stafseth (27) in 1938. He states, "The whole blood test has been used exclusively on the flock of the Michigan State College since 1931, and chick mortality due to pullorum disease has been practically eliminated. For several seasons we have failed to isolate pullorum bacilli from chicks sent to us from this flock for examination. These results have convinced us as to the value of this test."

Biely and Lloyd (2) in 1934 state that, "It is generally recognized and rightly so, that a single test, by any method will not detect all the infected birds in a flock. At least two or three tests in one season are required . . . . the whole blood agglutination test lends itself admirably to repetition of tests, since it is simple, easily applied, can be conducted on the farm at low cost, and can be performed at any time of the year with little disturbance to the birds."

In 1938–39, Shaw, Gwatkin, Moynihan, Traves and Roach (26) made comparative tests with the whole blood test and the single tube agglutination

test with a 1-50 dilution on 2,375 samples of chicken blood. This is hitherto unpublished work and is included herein with the permission of the authors. Diagnoses were made in two laboratories by the tube test. The results are tabulated in Table 1.

TABLE 1.—RESULTS OF COMPARATIVE DIAGNOSES

Whole blood test	Tube test		No. of birds
	Lab. 1	Lab. 2	
Positive	Positive	Positive	571
Negative	Negative	Negative	1363
Positive	Negative	Positive	287
Positive	Positive	Negative	7
Positive	Negative	Negative	99
Negative	Positive	Negative	14
Negative	Negative	Positive	34
			2 375

It will be seen that there was agreement in 1,934 diagnoses (81.43%) and disagreement in 441 (18.56%). The whole blood test and tube test in Lab. 1 agreed in 578 positives and 1,397 negatives, a total of 1,975 (83.15%), and disagreed on 400 (16.84%). The whole blood test and tube test in Lab. 2 agreed on 858 positives and 1,377 negatives, a total of 2,235 (94.1%) and disagreed on 140 (5.89%), while tube test Lab. 1 and tube test Lab. 2 agreed on 571 positives and 1,462 negatives, a total of 2,033 (85.6%) and disagreed on 342 (14.4%).

There was a greater agreement between the whole blood test and the tube test Lab. 2, than there was between the two laboratories.

Retests were made on one group of 420 birds. Many of these had not been tested previously by the tube test in Lab. 2, and a few birds were negative to all tests in previous comparisons. The results are shown in Table 2.

TABLE 2.—RETESTS OF BIRDS IN TABLE 1

Whole Blood test	Tube Test		No. of birds
	Lab. 1	Lab. 2	
Positive	Positive	Positive	174 *(a)*
Negative	Negative	Negative	21 *(a)*
Positive	Negative	Positive	49 *(b)*
Negative	Positive	Negative	21 *(b)*
Positive	Positive	Negative	59 *(c)*
Negative	Negative	Positive	5 *(c)*
Negative	Positive	Positive	5 *(d)*
Positive	Negative	Negative	86 *(d)*
			420

NOTE:

(a) All three tests agreed on 174 positive and 21 negatives (46.42%).

(b) Whole blood test and tube test Lab. 2, agree on 49 positives and 21 negatives diagnosed oppositely by the tube test Lab. 1 (16.66%).

(c) Whole blood test and tube test, Lab. 1, agree on 59 positives and 5 negatives diagnosed oppositely by tube test, Lab. 2, (15.23%).

(d) Tube tests Lab. 1 and Lab. 2, agree on 5 positives and 86 negatives diagnosed oppositely by the whole blood test (21.66%).

It will be seen that the whole blood test removed 86 birds that were negative to the tube tests. This, however, is much less serious than the failure to recognize positive birds, and whereas the whole blood test left in 5 positives, the tube test Lab. 1, left in 49, and the tube test Lab. 2, left in 59 samples which were positive by the other tube test and the whole blood test.

COMPARATIVE TESTS IN 1939-40

In view of the results of the 1938–39 work, arrangements were made to carry on another comparative testing program in 1939–40, and this paper reports the results of this work.

Technique and Methods

Tube Test

S. pullorum No. 4011, was grown for 48 hours at 37° C. on beef infusion agar. The growth was collected in concentrated form with 0.9% sodium chloride solution containing 0.5% phenol. It was stored in the refrigerator and diluted for use with the same phenolized saline to a density equal to No. 1 tube of McFarland's nephelometer. Just before use, 0.5% of normal sodium hydroxide solution was added. One tube, 100 × 13 mm. was used for each sample and 0.04 cc. of serum was used with 2.0 cc. of antigen, making a dilution of 1-50. Positive and negative controls were set up with each lot of samples, which were incubated for 24 hrs. at 37° C. and overnight at room temperature. The pipettes used for the serum were 0.2 cc. graduated in 0.02 cc., and the antigen pipettes were 10 cc. graduated in 1.0 cc. Samples were set up the day received or when necessary were held in the refrigerator until the following day. All the tube testing was done by one of the authors (I.W.M.).

Whole Blood Test

Lederle pullorum disease antigen (stained), produced under U.S. Patent No. 1,816,026 was used. The technique used was that suggested by Biely and Roach (3) in which glazed spot plates with sunken wells were substituted for flat glass plates as advocated by many investigators. Antigen was distributed in the wells by the droppers provided and blood was measured with the loop supplied with each separate bottle of antigen. Spot plates were kept at temperature from 70° F. to 80° F. by use of cans of hot water. Readings were made as soon as possible, but no reaction requiring more than 2 minutes to develop was recorded as positive.

Reading Tests

While in practice it is usual to regard any degree of reaction in a dilution of 1-50 as positive, it was decided to record both the tube test and whole blood test as ++, +, ? and −. This represented in the former test a typical flocculent reaction, a fine clumping, partial, and negative; and in the latter typical rapid reaction, slow reaction, partial, and negative.

Blood samples for the laboratory were collected at the time the whole blood tests were made and shipped by express the same night. The whole blood test was under the direct supervision of one of the authors (C.W.T.).

Conditions were controlled as carefully as possible and results of both tests were sent to a referee (R.G.) and were not released until the results of both tests had been received. This was done to remove any possibility

of one set of results influencing the other. The comparative results were not made known to the whole blood or tube test authorities until reports of both tests were in the hands of the referee. Comparative tests were made in two groups, which were reported separately.

First Group

The first group consisted of 13 flocks, the birds in 7 of which were tested twice by both methods, with the exception of those that had been removed or died between tests, and haemolyzed samples. In all, 4,146 tests were made during the winter months.

The field work was carried on by 4 men under weather conditions when the outside temperature varied from 70° F. to −25° F. In the extreme cold, wherever possible, the poultry houses were warmed by oil stoves to maintain comfortable working temperatures. Various conditions were encountered as far as the flocks themselves were concerned, some being kept under ideal conditions while others left much to be desired.

No attempt was made in the laboratory to test samples that were haemolyzed or spoiled by frost, as it is not possible to obtain clear-cut results from such material. The percentage of spoiled samples was high, amounting to 6.84%, but this is a common occurrence where testing is done in areas where weather conditions and transportation facilities make the satisfactory shipment of blood samples a matter of considerable difficulty.

Omitting all spoiled samples and considering questionable reactions by either test as positives, which is the practice in routine work, there was an agreement on the samples tested by both methods of 92.41%. Of these samples, 87.43% were negative by both tests, so the agreement among the samples showing some degree of reaction in one test or the other, was very much less, but from a practical standpoint, it appears reasonable to base the percentage agreement on the total. The diagnoses on the total samples on which comparisons could be made, are given in Table 3.

Table 3—Diagnoses in 3,861 samples tested by both methods

	Both −	Both +	Both ?	WB+ T−	WB− T+	WB+ T?	WB ? T+	WB− T?	WB ? T−	Total
1st test	2,613	97	2	73	53	6	2	68	12	2,926
2nd test	763	83	—	51	15	7	1	7	8	935
	3,376	180	2	124	68	13	3	75	20	3,861

The second test showed conclusively that 49 of the differences in Table 3 were due to errors in the tube test. In one flock (*c*), this test showed 19 positives and 30 suspicious. This proportion of suspicious to positive samples appeared obviously wrong at the time, but as the test and the sera had been discarded when the report was received, nothing could be done about it. The agreement between the two tube tests was

only 50% and the agreement between the first whole blood and tube tests was also only 50%. The agreement between the two methods on the second test was 94.7%. Details of both tests are shown in Table 4.

TABLE 4.—DETAILS OF BOTH TESTS IN FLOCK C

	Both −	Both +	Both ?	WB+ T−	WB− T+	WB+ T ?	WB ? T+	WB− T ?	WB ? T−	Total
1st test	48	2	—	—	19	—	—	30	—	99
2nd test	88	1	—	2	—	1	—	—	3	95
	136	3	—	2	19	1	—	30	3	194

Table 4 clearly shows that in Table 3 (5th column) 19 of the differences may be ascribed to the tube test and that in the same table (8th column), 30 may be ascribed to the same source. While we are unable to account for such an error, it is recorded in order to present an unbiased report on the difference in the two methods.

On several occasions, field and laboratory reports had notations to the effect that reactions were difficult to read, and this was evidenced by the fact that a fifth symbol (−?) crept into many reports, even though instructions were that all diagnoses were to be recorded as ++, +, ?, or −, indicating that there were many reactions that might be termed "marginal".

Agreement Between Total Number of Tests Performed and Constancy Between First and Second Tests by Each Method

As already stated, for various reasons a number of birds were tested once only by both methods. Diagnoses on these are shown in Table 5.

TABLE 5.—BIRDS TESTED ONCE BY BOTH METHODS

Whole blood test	Tube test	No. of birds
Positive	Positive	28
Negative	Negative	1,908
Negative	Positive	43
Positive	Negative	38
Total		2,017

Diagnoses made by both methods on two tests are shown in Table 6.

The whole blood test was constant in 827 readings (89.79%) on first and second test, as shown in *a*, *c*, and *d*, of Table 6, and disagreed on 94 diagnoses (10.2%), as shown in *b*, *e*, and *f*.

The tube test agreed on 802 readings (87.07%) on first and second test, as shown in *a*, *c*, and *e* of Table 6, and disagreed on 120 or 12.9% as shown under *b*, *d*, and *f*.

TABLE 6.—DIAGNOSES ON TWO TESTS

1st test		2nd test		No. of birds			
Whole blood	Tube test	Whole blood	Tube test				
Positive	Positive	Positive	Positive	68			
Negative	Negative	Negative	Negative	652			
				—	720	(78.15%)	*(a)*
Negative	Negative	Positive	Positive	13			
Positive	Positive	Negative	Negative	8			
				—	21	(2.26%)	*(b)*
Positive	Negative	Positive	Negative	15			
Negative	Positive	Negative	Positive	2			
				—	17	(1.84%)	*(c)*
Positive	Positive	Positive	Negative	5			
Positive	Negative	Positive	Positive	3			
Negative	Positive	Negative	Negative	65			
Negative	Negative	Negative	Positive	18			
				—	91	(9.77%)	*(d)*
Negative	Positive	Positive	Positive	5			
Positive	Negative	Negative	Negative	29			
Negative	Negative	Positive	Negative	31			
				—	65	(7.05%)	*(e)*
Positive	Negative	Negative	Positive	2			
Negative	Positive	Positive	Negative	6			
				—	8	(0.86%)	*(f)*
Total					922		

NOTE:
(a) Diagnoses constant by both methods and in agreement on both tests.
(b) Diagnoses changed by both methods and in agreement on both tests.
(c) Diagnoses constant but in disagreement on both tests.
(d) Diagnoses constant by whole blood but not by tube test on both tests.
(e) Diagnoses constant by tube test but not by whole blood test on both tests.
(f) Diagnoses changed by both methods from first to second test.

The constancy of diagnosis is calculated on the number of birds tested by both methods twice, rather than on the number tested by each method twice, as it makes a more accurate comparison of constancy of diagnosis.

As noted previously, these samples had been recorded as positive, questionable and negative, but in order to obtain figures for percentage agreement and for constancy of diagnosis, questionable readings were grouped in Tables 5 and 6 with the positives, as would be done in actual practice. In this group of birds, the whole blood was 2.72% closer in constancy of diagnosis between the two tests than was the tube test.

SECOND GROUP

This group was part of the flock at an Institution in the Province of Alberta.

On the first test, 1,720 blood samples were taken between May 13 and May 18, 1940, and sent to the Veterinary Research Station for the tube test. Whole blood tests were conducted at the time the samples were collected. Through a misunderstanding, four boxes containing 334 samples were spoiled in transit. Of the remaining 1,386 samples, 60 were

haemolyzed or jellied when received at the laboratory, and 2 samples congealed in the whole blood test and no diagnoses were made. Consequently comparisons were made between 1,324 diagnoses by the whole blood and tube tests. The readings were recorded as positive or negative only. The diagnoses on these samples are shown in Table 7.

TABLE 7.—RESULTS OF FIRST TEST ON SECOND GROUP

Whole blood test	Tube test	No. of birds
Positive	Positive	213
Negative	Negative	1,034
Positive	Negative	71
Negative	Positive	6
Total		1,324

The two tests agreed on 213 positives and 1,034 negatives, a total of 1,247 or 94.18%. Disagreements were recorded on 77 blood samples or 5.81%. Of these, 73 were positive to the whole blood test and negative to the tube test, while 6 were positive to the tube and negative to the whole blood method.

The whole blood diagnoses on samples spoiled in transit, or haemolyzed or jellied when received in the laboratory, were 80 positive and 312 negative, and two were jellied.

Thus, on the flock, the whole blood test diagnosed 21.27% as positive and 78.72% as negative (1,716 birds), while the tube test diagnosed 16.46% as positive and 83.50% as negative (1,324 birds).

The second test on this flock was made by the whole blood test from June 10 to June 13, and blood samples were sent to the laboratory each day. On this test, readings were made on 1,658 samples by both methods, with the results as shown in Table 8.

TABLE 8.—RESULTS OF SECOND TEST ON SECOND GROUP

Whole blood test	Tube test	No. of birds
Positive	Positive	285
Negative	Negative	1,268
Positive	Negative	92
Negative	Positive	13
Total		1,658

As will be seen, 285 were positive and 1,268 negative to both tests, a total of 1,553 or an agreement of 93.66%, while 92 were positive to the whole blood and negative to the tube, and 13 were negative to the whole blood and positive to the tube test, a total of 105 or 6.33%.

In all, comparisons were made on 2,982 samples and the agreement between the two methods of testing was 498 positive and 2,302 negative, a total of 2,800 or 93.89%.

The mere recording of agreement and disagreement between methods is no measure of the comparative accuracy or otherwise of either method nor of the relative efficiency of those employed in the testing. The consistency of diagnosis from one test to another by either method is of importance. Table 9 shows the diagnoses made by both tests and both methods.

TABLE 9.—DIAGNOSES BY BOTH METHODS ON TWO TESTS

1st test		2nd test								
W.B. test		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	NT.	Dead	Total
	Tube test	Pos.	Neg.	Neg.	Pos.	NT.	NT.	Neg.		1st test
Pos.	Pos.	203	—	3 (b)	2 (a)	—	—	—	5	213
Neg.	Neg.	12 (ab)	947	33 (a)	5 (b)	—	8	—	29	1,034
Pos.	Neg.	8 (b)	16 (a)	43	—	2	—	—	2	71
Neg.	Pos.	3 (a)	1 (b)	—	2	—	—	—	—	6
Pos.	NT.	55	7 (a)	11	1 (a)	2	—	1	3	80
Neg.	NT.	4 (a)	293	2 (a)	3	—	4	—	4	310
NT.	NT.	2	—	—	—	—	—	—	—	2
NT.	NT.	2	—	—	—	—	—	—	—	2
Total—2nd test		285	1,268	92	13	4	12	1	43	1,718
						Omitted (clerical error on 2nd test)				2
										1,720

NOTE:
NT.—Not tested (blood samples spoiled).
(a) Changes made by whole blood test from 1st to 2nd test.
(b) Changes made by tube test from 1st to 2nd test.

A study of Table 9 shows that the whole blood changed 54 diagnoses from negative to positive and 26 from positive to negative from the first to the second test. A total of 1,670 birds were tested twice by this method, which gives a constancy of diagnosis of 95.2%. The tube test changed 25 from negative to positive and 4 from positive to negative. Since 1,267 birds were tested twice by this method, the constancy of diagnosis was 97.7%.

Thus there was a 2.5% greater constancy of diagnosis with the tube test than with the whole blood. This 2.5% advantage over the whole

blood method would, in routine testing, have been nullified to some extent by the number of spoiled samples which could not be tested in the laboratory

Table 9 further shows that both methods changed 12 diagnoses from negative to positive on the second test: that 55 samples which were unfit for testing by the tube test on the first test, were reported as positive by the whole blood, and that these diagnoses were confirmed by both whole blood and tube on the second test; that 2 birds were positive to the tube and negative to the whole blood test on both occasions, while 43 were positive to the whole blood and negative to the tube on both tests.

The whole blood changed 3 negatives to positive and 16 positives to negative and thus came into agreement with the tube diagnoses, while the tube test changed 8 negatives to positive and one positive to negative to agree with the whole blood test.

Table 9 shows that the tests in agreement represented a high percentage of the total, but it should be pointed out that the whole blood method on each test showed more positives than the tube test.

Post-mortem and Bacteriological Examination on 17 Birds in the Second Series

The management would not agree to a third handling of the flock so that 17 birds selected for examination were removed when the second test was carried out, at which time the second tube test had not been made, although it was later available for incorporation in the results.

The birds were shipped alive to the Veterinary Research Station, and post-mortem and bacteriological examination of each was made shortly after they were killed. A careful search of the abdominal cavity was made and any suspicious part, in addition to the ovary and oviduct, was cultured on brilliant green liver agar and beef infusion agar pH 7.8. The ovary was then removed to a flask of beef infusion broth of the same pH, from which platings on brilliant green liver agar were made at 24 hours. All plates were examined after 48 hours incubation. Brilliant green was added to the liver agar in a concentration of 1-75,000, which permitted good growth of a control culture of *S. pullorum*.

Twelve of the birds selected were positive by the whole blood and negative by the tube method on the first test, shown in Table 9. One of these (No. 17 in Table 10) was positive by the tube method on the second test. A third test at time of slaughter showed a complete reaction in 1-50 and partial in 1-100. This bird had a dry, powdery mass of material in the oviduct, from which a member of the coli-aerogenes group was isolated. Only one of these 12 birds (No. 12 in Table 10) yielded *S. pullorum*. This bird was positive by both whole blood tests and negative by both tube tests, and also negative by the latter method in all dilutions from 1-25 to 1-3200 at time of slaughter. There were no lesions and the organism was isolated from the brilliant green liver agar plate made from broth culture of ovary but not from direct plating of the ovary.

The other 5 birds were positive by the tube test but negative by the whole blood method. Three of these were positive by the second whole blood test and one of the tube reactions had become negative (No. 14 in Table 10). *S. pullorum* was recovered from this bird, the serum of which

at time of slaughter gave a partial reaction in 1-50 in the tube test. *S. pullorum* was recovered from another of these birds (No. 1 in Table 10) which was positive on both tube tests, negative by the first whole blood, but positive by the second one. It had well-marked lesions, the ova being the large brownish-green, angular type of the classical pullorum ovary.

All three cultures were typical in morphology, staining and cultural appearance. They were rapidly agglutinated in coarse floccules by pullorum antiserum but were not agglutinated by normal, colon, or any of the other sera tried. They produced acid and gas in dextrose and mannite but not in lactose and maltose. The agglutination reactions of these birds are given in Table 10.

TABLE 10.—AGGLUTINATION REACTIONS OF 17 BIRDS EXAMINED FOR *Salmonella pullorum*

No.	Whole blood		Tube		Tube test at time of P.M.								Cultures
	1st	2nd	1st	2nd	1-25	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	
1	−	+	+	+	+	+	+	+	+	+	P	P	*S. pull.*
2	+	+	−	−	−	−	−	−	−	−	−	−	−
3	−	+	+	+	P	P	P	−	−	−	−	−	−
4	+	+	−	−	−	−	−	−	−	−	−	−	−
5	−	−	+	+	P	P	P	−	−	−	−	−	−
6	+	+	−	−	−	−	−	−	−	−	−	−	−
7	+	+	−	−	P	−	−	−	−	−	−	−	−
8	−	+	+	+	P	P	−	−	−	−	−	−	−
9	+	+	−	−	−	−	−	−	−	−	−	−	−
10	+	+	−	−	−	−	−	−	−	−	−	−	*S. pull.*
11	+	+	−	−	P	P	−	−	−	−	−	−	−
12	+	+	−	−	P	−	−	−	−	−	−	−	−
13	+	+	−	−	−	−	−	−	−	−	−	−	−
14	−	−	+	−	+	P	−	−	−	−	−	−	*S. pull.*
15	+	+	−	−	−	−	−	−	−	−	−	−	−
16	+	+	−	+	P	P	P	P	−	−	−	−	−
17	+	+	−	+	+	+	P	−	−	−	−	−	Coli-aero-genes

NOTE:
+ Complete agglutination.
P Partial agglutination.
− No agglutination.

S. pullorum was recovered from Nos. 1 and 14 by direct plating but only on the plate made from broth culture with No. 10, and even on that the growth was not as heavy as on the direct plates of the other two. This bird failed to give a tube reaction on any of the tube tests, though the whole blood test twice recorded positive diagnoses. Bird No. 14 failed to react by the tube method on the second test. The organism was thus recovered from one of 12 birds that were positive only by the whole blood method on the first test (8.3%), and from 2 out of 5 that were positive only by the tube method on the first test (40%).

It should be noted that while the whole blood testing was done by four men with the first group of birds covered in this report, the two tests by the whole blood method on the second group of birds, were done by one man.

ADVANTAGES OF THE WHOLE BLOOD METHOD

The following figures were computed by the Alberta Provincial Department of Agriculture, at the conclusion of the first year (1939–40) with the whole blood test during which time 102,522 birds were tested. The flocks tested averaged 201 birds per flock.

Testing in 60 flocks was stopped when a heavy reaction was found. With the tube test, all of the birds in each of these flocks would have been tested, with an estimated cost of $600.00 to the owners.

Since reactors are removed as testing with the whole blood test is conducted, there is no need for a second handling of the flock. Savings in labour to owners at $1.00 per day in handling the birds a second time, on the flocks tested, is estimated at $518.00: loss in production from handling (2 eggs per bird at 10 cts. per dozen) equals $1,500.00, and the difference of $1\frac{1}{2}$ cts. in cost between tube and whole blood tests, is a saving of $1,537.00.

There was an estimated saving to the owners of $4,150.00. In fairness, there should be some deduction from this for birds removed as positive, which should probably have remained in the flock. This figure, however, is negligible compared to the gross savings.

Savings to the Poultry Branch of the Provincial Department of Agriculture, amounted to $3,500.00 for fees which had been previously used for laboratory charges, $600.00 express on blood samples and return of empty blocks, and $300.00 in wages. Against this, is $150.00 for antigen and $50.00 for upkeep of equipment, leaving a saving to the Department of $4,200.00.

Weather conditions and transportation facilities and schedules are such that the high percentage of spoiled samples reported herein is the usual occurrence. In the 1939-40 blood testing season, two R.O.P. flocks had to be bled a second time on account of haemolyzed samples on the first test, and two flocks had to be bled a third time because the second test samples, required on account of a reaction over 4% on the first test, were frozen. This difficulty has been obviated by the use of the whole blood test.

It should be emphasized, however, that experience is as necessary with the whole blood test, as with any other tests, if satisfactory results are to be obtained, and the use of the test by inexperienced persons can only result in trouble for all concerned. This is well illustrated by Biely and Roach (3) in 1932. They obtained an agreement of 98.3% between the whole blood and serum plate tests when trained technicians were employed, whereas there was a difference of 12% when untrained men were purposely put on the whole blood test.

SUMMARY

Comparative tests for *Salmonella pullorum* by the whole blood and tube agglutination tests were conducted on two groups of birds in 1939–40.

There were 2,926 diagnoses made on the birds in 13 flocks in Group 1, and retests were made on 935 birds in 7 flocks. A total of 3,861 diagnoses were made by both tests, with an agreement of 92.41% by both methods.

Constancy of diagnosis from 1st to 2nd tests, was 89.79% for the whole blood test and 87.07% for the tube test, there being a greater constancy of diagnosis of 2.72% by the whole blood test.

In the second group, 1,324 tests were made by whole blood and tube tests, there being an agreement of 94.18%. Retests were made on 1,658 birds, (some were not tested by the tube method on first test on account of spoiled samples), and an agreement of 93.66% was secured. The combined diagnosis of both tests by both methods showed an agreement of 93.89%. Retests on 1,670 birds by the whole blood test gave a constancy of diagnosis of 95.2%, while retests on 1,267 birds by the tube method, gave a constancy of diagnosis of 97.7%, there being a greater constancy of diagnosis of 2.5% in favour of the tube test.

The whole blood test showed more positives than the tube method in each group.

Bacteriological examination of 12 birds positive by the whole blood method only, on the first test, yielded *S. pullorum* from 1 bird (8.3%), while the organism was recovered from 2 out of 5 (40%) that were positive to the tube only. A member of the coli-aerogenes group was isolated from one bird positive to the whole blood test only.

The results obtained would suggest that the whole blood test in competent hands could be used in a program of pullorum control, with results equally as satisfactory as those obtained by the tube method, under the weather conditions and transportation difficulties often prevailing during the testing season in Alberta. The whole blood test has the advantage of being more economical in its application.

ACKNOWLEDGMENTS

The authors are indebted to the late Hon. D. B. Mullen, Minister of Agriculture, Province of Alberta and to Dr. E. A. Watson, Dominion Animal Pathologist, for their advice and assistance with this project, and are deeply grateful to them for the opportunity of carrying it out.

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METHODS AND RATIONS FOR FATTENING POULTRY[1]

III. THE EFFECTS OF VARIOUS FATS, NUMBER OF FEEDINGS AND LENGTH OF FATTENING PERIOD

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[Received for publication June 18, 1940]

The work reported is a continuation of research on the fattening of poultry, some results of which have been published previously (3, 4). Corn having been shown to be the most satisfactory grain of those tested (loc. cit.) it was determined to proceed from that point using various supplements to a ground yellow corn ration. Since there are many references in the earlier non-technical literature to the value of various fats for fattening poultry it was decided to investigate the efficiency of such feeds. At the same time the experiment was so arranged that information relative to two of the most controversial matters of fattening procedure, namely the number of feedings daily and the length of fattening period, would be available.

The technical literature covering these matters is relatively meagre. Although reference is made to fats and the feeding methods under consideration frequently in the older literature, such as Wright's Book of Poultry, the deductions are based largely on experience rather than controlled experiment. In recent years Maw *et al* (6) found that the addition of corn oil at levels varying from $2\frac{1}{2}$ to 10% to a basal ration of wheat, oats, and barley caused no significant differences in gain over the basal ration. They were also able to produce greater gains during 14- and 21-day fattening periods than during one 7-day period. Gutteridge (4) found that the addition of 5% of mutton fat to a cereal ration materially increased gain, deposition of fat, and grade of the dressed carcass. As to quality of fat, Cruickshank (1, 2) stated that the nature of the substance fed was important in determining the hardness of the fat, and that the fat synthesized from carbohydrates was relatively soft. As a result of work with corn Cruickshank was of the opinion, based on iodine values, that corn has no detrimental effect in comparison with other cereals on softness of fat. Hilditch (5) found the digestibility of fats to be almost entirely dependent upon whether they were fluid or otherwise at body temperature.

Controlled experimental work to determine whether 2, 3, or 5 feedings daily are most satisfactory was carried out by Maw and Maw (7). They found 3 and 5 feedings daily to give greater gains than 2 feedings daily.

MATERIALS AND METHODS

A total of 180 Barred Plymouth Rock cockerels were used in these tests. In order to obtain individual feed consumption records, laying cage batteries were used. The birds were randomized according to hatch, battery, tier in the battery, ration and treatment, and were fed as follows:

[1] Contribution from Poultry Division, Central Experimental Farms Service, Dominion Department of Agriculture, Ottawa, Canada.
[2] Agricultural Scientist (Nutrition).
[3] Agricultural Assistant.

Group A—Ground yellow corn including 10% mutton fat.
Group B—Ground yellow corn including 10% beef fat.
Group C—Ground yellow corn including 10% corn oil.
Group D—Ground yellow corn including 10% mutton fat, 10% meat meal and 5% ground oyster shell.
Group E—Normal range rearing.

All fattening feeds were mixed with skim milk. The animal fats were rendered and mixed quickly while hot with the ground grain to avoid lumping.

One-third of the birds of each of the above groups was starved and killed after 1 week, 2 weeks and 3 weeks fattening to determine the effect of length of fattening period. One-half of each of these groups received feed twice daily at 7 a.m. and 5 p.m. while the other half were fed 3 times daily at 7 a.m., 12 noon, and 5 p.m. All unconsumed feed was weighed back after a 20-minute feeding period.

Group E received no fattening treatment but remained on range with growing mash ad lib. and scratch grain night and morning. One third of these birds were also starved and killed after 1-, 2-, and 3-week feeding periods.

The experiment was planned and carried out so as to be amenable to analysis by the methods of variance and covariance. The criteria of efficiency of the various feeds and treatments were gain in body weight, increase in percentage fat, colour and firmness of fat (A, B, C and E groups only), and market grading of the dressed carcass. Increase in percentage fat was determined by sampling the live bird before fattening and the dressed carcass after fattening according to a technique described elsewhere (4). Relevant data used in the variance and covariance analyses were body weight gain, feed consumption, initial percentage fat, increase in percentage fat, and initial body weight. The gains and increase in percentage fat were adjusted for the effect of the other correlated variables just mentioned so as to express both gain and increase in percentage fat on a basis of equal initial body weight, feed consumption and initial percentage fat. The method of adjustment by the partial regressions of the correlated variables was used. A high correlation between feed consumption and gain at .6701 and between initial percentage fat and increase in percentage fat at $-.5525$ was recorded with correspondingly high regressions which had the greatest effect upon the adjustment both of variance and of means. Since the adjustment of treatment means makes these means no longer independent it becomes necessary to make appropriate correction not to the stardard error of the means themselves but to the standard error of the difference between means. This entailed the extension of Wishart's formula for 2 independent variables to 4 (8). The extensive statistical analyses including tables of variance and covariance and the necessary adjustments to the mean squares will not be shown in this paper.

RESULTS

Tables 1 and 2 show the weight gains and increases in percentage fat respectively, which were made with the various rations and treatments.

TABLE 1.—OBSERVED AND ADJUSTED MEANS—GAIN IN BODY WEIGHT (GRAMS)

Treatment	Observed mean			Adjusted mean		
	1 wk.	2 wk.	3 wk.	1 wk.	2 wk.	3 wk.
A—Mutton fat	240.33	493.83	638.00	257.25	534.60	598.66
B—Beef fat	338.00	549.50	555.58	301.69	493.52	494.26
C—Corn oil	319.67	579.08	455.58	316.85	543.35	458.85
D—Complex ration	226.08	365.58	373.75	248.36	416.46	471.16
E—Range	79.42	186.87	227.67	—	—	—
2 feedings daily	285.67	477.83	512.75	298.76	517.60	568.77
3 feedings daily	275.38	516.17	498.71	262.26	476.40	442.68
1-week feeding*	281.02			652.48		
2-week feeding*		497.00			459.45	
3-week feeding*			505.73			171.90

*Exclusive of range group. Necessary difference at 19 : 1—feeds 129.0; feedings 47.4; weeks 58.0 (observed means only).

TABLE 2.—OBSERVED AND ADJUSTED MEANS—INCREASE IN PERCENTAGE FAT

Treatment	Observed mean			Adjusted mean		
	1 wk.	2 wk.	3 wk.	1 wk.	2 wk.	3 wk.
A—Mutton fat	19.01	22.33	33.56	19.65	22.73	34.98
B—Beef fat	23.65	22.15	32.46	22.83	22.11	30.71
C—Corn oil	19.93	25.91	31.38	19.83	26.11	30.18
D—Complex ration	18.43	18.99	24.03	18.72	18.43	25.58
E—Range	6.28	5.90	17.96	—	—	—
2 feedings daily	20.77	21.72	30.84	19.76	21.71	30.21
3 feedings daily	19.74	22.90	29.88	20.74	22.91	30.51
1-week feeding*	20.25			23.75		
2-week feeding*		22.35			21.88	
3-week feeding*			30.36			27.34

*Exclusive of range group. Necessary difference at 19 : 1—feeds 5.47; feedings 2.00; weeks 2.47 (observed means only).

Examination of Tables 1 and 2 on the basis of significant differences indicates striking effects in several instances. Range feeding was inferior to fattening in crates even where the poorest crate fattening ration, namely the complex, was concerned. For example, the total gain in 3-weeks range feeding just equals the 1-week gain of the poorest fattened group, while the increase in percentage fat fails to equal that for 1 week in any group. Obviously range feeding was very uneconomical in comparison. Furthermore the complex ration was inferior to the other fattening rations fed, although since the meat meal used was subsequently found to be contaminated and relatively unpalatable, this is not considered to be a true result and it must not therefore be considered from these data that this combination of feeds is necessarily unsatisfactory. This group is therefore omitted from the discussion which follows.

Gain in body weight

Considering the observed mean gains in Table 1 over a 3-week fattening period the gains were significantly greater with mutton fat than with corn oil, the latter being actually only 69% of the former. When adjustment is made for varying levels of feed consumption and other related factors, i.e., on the basis of equal feed consumption, starting weight, initial percentage fat and increase in percentage fat, the gain for the corn oil group becomes 76% of that for the mutton fat group. There was apparently a falling off in gains with corn oil during the third week of fattening, whereas the gain on mutton fat was well maintained. On the basis of adjusted gains the mutton fat group was also superior to the beef fat group, indicating a tendency to more efficient maintenance of gains than either beef fat or corn oil over the longer fattening period. Actually beef fat produced negligible gains during the third week while the corn oil group lost weight on the average.

Considering the effect of 2 feedings as against 3 feedings daily no significant differences are apparent although the adjusted means indicate a superiority in efficiency for 2 feedings daily over the 3-week period, the gain on 3 feedings being only 76% of that for 2 feedings.

With respect to length of fattening period, 2-week fattening produced much greater gains than did 1-week fattening, the latter being only 57% of the former. This, of course, is to be expected for a longer period providing that good gains are being made. Three weeks of fattening, however, produced only a relatively small gain over 2 weeks fattening, the one being 98% of the other. Obviously the third week of fattening gave almost negative results and was definitely uneconomical. The adjusted gain made is interesting in that it shows a very great efficiency of gain during the first week of fattening, approximately 70% of that efficiency for the 2-week period, and only 26% of the first week's efficiency during the 3-week fattening period. Similarly the 3-week period was only 37% as efficient as the 2-week period.

Increase in percentage fat

In increase in percentage fat there were no significant differences between feeds either in observed or adjusted means. There was however, a differential reaction to length of feeding period for the different feeds as indicated by a fairly uniform increase in percentage fat from week to week for the corn oil group, whereas that for the other feeds was negligible from 1 to 2 weeks but was very great from 2 to 3 weeks.

Between 2 and 3 feedings daily no differences in fat increase were apparent.

Length of feeding period shows highly significant differences between the increase in fat for 3 weeks feeding over that for both 1 and 2 weeks feeding, both as to actual and adjusted increase. It should be particularly noted that this applies to the group on range as well as to the crate fattened birds, and since the environment and reactions of this group were otherwise vastly different from that of the crate fattened birds it is presumed that this sudden increase was spontaneous and governed by factors entirely apart from the treatments of this experiment. This matter is under further investigation.

Colour and firmness of fat

Colour of fat is of economic importance because of the price differential paid in Canada for white fleshed birds (milk fed classes) and on account of the preference for white flesh on the British market. Examination of the melted fats showed great variability between individual birds in all groups and very definite differences between groups. The range fed group which were on alfalfa fields ranged from deep yellow to orange, the corn oil birds from lemon yellow to deep yellow, and the mutton and beef fat birds from pale whitish yellow to lemon yellow. Because of the variety of pigments involved in the fat comparison it was not feasible to classify properly the different groups on the basis of tintometer readings, but the differences above noted were sufficiently marked to permit of the conclusions just drawn.

To determine the best measure of consistency of fat, the relative suitability of iodine numbers (Harris), refractive indices (Butyro-refractometer at 40° C.), and melting points (Capillary tube) were investigated. Since a measure of the softness or hardness of the fats was desired, largely because of a preference for the appearance of hard fatted birds, it was finally decided that the physical measurement of melting point was most suited to express the apparent condition of the fat. A variance analysis covering the fats of 12 birds on each of mutton, beef fat, corn oil, and range treatments showed a remarkably small variability within groups with means as follows:—Mutton fat 40.6° C.; beef fat 40.3° C.; corn oil 25.1° C.; range fed 34.8° C. With a necessary difference for significance at the 5% point of only .455° C. it may be concluded that mutton fat and beef fat produce fats of equal hardness, the carbohydrate ration of the range fed birds slightly softer fat (approximately 15% lower melting point), and corn oil very soft fat. It should be noted that some fat was found free in liquid form in the abdomen of some of the corn oil fed birds. It is also of interest that at a 10% level of feeding the mutton fat imparted its flavour to the meat. Since 5% of mutton fat has previously been reported (4) to give excellent gains with no mutton flavour in the chicken a level somewhere between 5% and 10% would presumably be most satisfactory.

Grading of the birds

All the cockerels were graded by a government grader according to Canadian Government Standards. Since degree of fatness of the carcass was the most important consideration in so far as the efficiency of the feeds and treatments was concerned, colour of flesh, blemishes, pin feathers, etc., were not considered as degrading, and the main consideration was whether the bird was sufficiently fat to make A or B grade. Table 3 indicates the actual grading.

It must be considered that this grading is necessarily a less accurate method of appraising the degree of fatness from the standpoint of the efficiency of the experimental treatment than the increase in percentage fat previously stated. Such a grading does, on the other hand, translate the treatment effects into their practical value in so far as grade is concerned. Mutton tallow and beef fat were definitely outstanding in that practically all birds were placed in grade A. Corn oil was inferior largely

TABLE 3.—GRADING OF THE DRESSED CARCASSES

	Feed					Length of fattening period*			No. of feedings*	
	Mutton fat	Beef fat	Corn oil	Complex ration	Range	1 wk.	2 wk.	3 wk.	2 feedings daily	3 feedings daily
	%	%	%	%	%	%	%	%	%	%
Grade A	91.4	94.4	73.5	69.4	19.4	70.2	93.5	83.3	78.6	85.9
Grade B	8.6	5.6	26.5	30.6	80.6	29.8	6.5	16.7	21.4	14.1

*Exclusive of range group.

due to consistency of fat and to losses sustained during the third week of fattening. It is worthy of note that only 19.7% of the range reared birds were capable of entering grade A. One-week fattening was definitely not sufficient to take full advantage of the grading possibilities, with 2 weeks being very satisfactory and 3 weeks actually poorer because of loss of weight and condition in some birds. Because of gains and increase in percentage fat previously reported, the difference in grading between 2 and 3 feedings daily of 7.3% is difficult to explain although it may well be a chance occurrence within the limits of experimental error.

DISCUSSION

These data have demonstrated very definitely that normal range feeding of roaster age cockerels is productive of very small gains in both body weight and fat and does not compare at all favourably even with the least satisfactory treatment in fattening cages in this experiment. The difference in favour of crate fattening is sufficient to far out-weigh the added labour and equipment necessary to carry it out successfully.

The two animal fats used in this test, namely beef fat and mutton fat, gave very satisfactory results both in gain and increase in fat and showed a tendency to superiority over corn oil. The superiority was particularly noticeable where the longer 3-week fattening period was used, in which the corn oil was less efficient. The excess softness of fat produced by corn oil was apparent on observation, through the lower grading of the birds and the lower melting point of the fat. That the quality of fat produced by mutton and beef fat was highly desirable was shown by the high melting point of the firm chicken fat produced and by the excellent grading of these birds. The fact that the mutton fat imparted its flavour to the chicken fat to some degree and that free fluid fat was found in the abdomen of some corn oil fattened birds may be taken to indicate a probable too high level of feeding when these fats were used at a level of 10% of the mash, dry weight. Differences between mutton fat and beef fat were negligible on the average. It would seem to be apparent from these data that confinement from green range, feeding of milk-mixed feeds, and the inclusion of fat in the ration each had the effect of decreasing the intensity of yellow pigment of fat laid down.

These data also indicate that there is no choice between 2 and 3 feedings daily, and it may be presumed therefore that the additional labour entailed in feeding 3 times daily would not be in any way justified.

Gains, increase in percentage fat, and the grading of the dressed carcasses indicated 2-week fattening to be superior to 1 week, whereas 3-week fattening was not justified in any instance. It would seem to be highly probable that the point of greatest efficiency might be 2 weeks plus or minus 2 or 3 days. In this connection it should be pointed out that the stock used in these tests was well fed and grown throughout the rearing period, and may be considered to have been in average good condition at the beginning of the fattening period. It is quite probable, therefore, that stock which was poorly reared might not react similarly to this stock where length of feeding period is concerned, and longer periods might give economical results under these circumstances.

The grading of the birds in this experiment was a fairly direct reflection of the actual gain and fat increases made and indicates the economy of proper fattening procedures for the production of quality stock as well as for the actual weight gained.

SUMMARY

1. Crate fattening was greatly superior to ordinary range rearing methods for the production of gain, increase in fat and grading of the dressed bird.

2. Beef fat and mutton fat were very satisfactory additions to the fattening mash, giving excellent gains, fat increase, and market grade, and producing a fat of desirable firm texture. A level of 10% of mutton fat had a tendency to impart its flavour to the fat of the bird.

3. Corn oil, while giving very satisfactory gains and fat increase, did not maintain either gains or increase for as long a period, and a fat was produced which was decidedly soft even to the extent of producing some free fluid fat in the abdomen of some birds.

4. In crate fattening 2 feedings daily was as efficient as 3 feedings for weight gain and fat increase.

5. With well reared cockerels of roaster age, 2 weeks of fattening gave greater and more economical gains and fat increase than 1 week, but 3 weeks of fattening was not justified by the results obtained.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the co-operation of A. Langlois and A. R. G. Emslie of the Division of Chemistry, Science Service, in the analysis of fat samples and the determination of certain physical measurements for these fats.

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A NOTE ON THE CALCULATION OF STANDARD ERRORS FOR TREATMENT MEANS AFTER ADJUSTMENT FOR REGRESSION[1]

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[Received for publication June 18, 1940]

The adjustment of error variance for the effect of correlated variables is accepted practice in dealing with projects which have been so planned as to be amenable to covariance analysis. Similarly, treatment means are adjusted by regression and these means must then be compared on a basis of standard errors. Fisher (2), Yates (6), Brady (1) and others have demonstrated techniques for the correction of the mean square made necessary because of non-orthogonality brought about by the loss of degrees of freedom for the regressions calculated. Since adjustment of the treatment means makes these no longer independent it also becomes necessary to calculate an appropriate standard error for differences between treatment means. Wishart and Sanders (4) have dealt with the case of one independent variable and Wishart (5) has extended the formula to cover two independent variables. Since the writers have had occasion, in the course of the analysis of data from fattening tests (3), to extend the correction to the case of 4 independent variables it was felt worthwhile to record the extended calculation for the benefit of investigators who may wish to follow out the technique.

The theory upon which the calculation of the estimated variance of corrected treatment differences is based has been thoroughly expounded by Wishart (5). As the number of independent variables increase so also do the formulae of the partial regression coefficients become more complex and their solution more onerous. The calculations of the determinants and their co-factors are consequently necessarily more complicated, actually running into numbers of 18 cyphers in our data with 4 independent variables. It will be noted that the formula cited is for the variance of the treatment differences rather than of the means themselves and is as follows: (Wishart's notation)

$$s^2\left\{\frac{2}{r}+\frac{\lambda_1^2A_{11}+\lambda_2^2A_{22}+\lambda_3^2A_{33}+\lambda_4^2A_{44}+2\lambda_1\lambda_2A_{12}+2\lambda_1\lambda_3A_{13}+2\lambda_1\lambda_4A_{14}+2\lambda_2\lambda_3A_{23}+2\lambda_2\lambda_4A_{24}+2\lambda_3\lambda_4A_{34}}{a_{11}A_{11}+a_{12}A_{12}+a_{13}A_{13}+a_{14}A_{14}}\right\}$$

Where s^2 = the error mean square in the analysis of residual variance after adjusting for regression

r = the number of observations for each group mean

λ_1, λ_2, etc. = the differences in the mean values of the independent variables X_1, X_2, etc. in the two groups under consideration

A_{11}, A_{12}, A_{22}, etc. = the cofactors of the elements a_{11}, a_{12}, a_{22}, etc. They are the solved determinants of the appropriate sums of squares and products from the error line of the variance and covariance analysis.

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For example A_{22} in the above formula is the determinant

$$+ \begin{vmatrix} a_{11} & a_{13} & a_{14} \\ a_{13} & a_{33} & a_{34} \\ a_{14} & a_{34} & a_{44} \end{vmatrix}$$

Where a_{11} is the sum of squares of the deviations of the x_1 variates from their mean, a_{33} the same for the x_3 variates and a_{13} the sum of the products of the deviations of x_1 and x_3 from their means etc., taken from the error line of the analysis of variance and covariance. The determinants involved are solved in the usual manner.

The formula is readily satisfied by substitution from the error line of the variance and covariance tables using the observed group means and the number of observations in each group as supporting data.

The t test now becomes applicable to the differences between corrected means as follows, using the data of the published paper (3) already referred to:—

Corrected mean of gain for group A — 510.428 gm.
Corrected mean of gain for group B — 401.166 gm.
Difference between corrected means — 109.262 gm.

Estimated variance of the corrected treatment difference

$$V(d) = 114.995 \quad sd = 10.724 \quad t = d/sd = \frac{109.262}{10.724} = 10.189$$

t at $p = 0.05$ for $n = 24$ (12 observations per group) $= 2.064$.

The difference between the mean gain of groups A and B of 109.262 gm. per bird is therefore significant.

ACKNOWLEDGMENT

The authors are indebted to Dr. John Wishart, Reader in Statistics, University of Cambridge, England for assistance in the extension of the formula here reported and this co-operation is gratefully acknowledged.

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